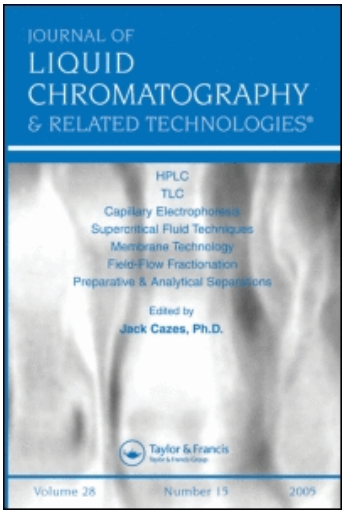

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 02 March 2003

To cite this Article Sakata, Masayo , Matsumoto, Kouchi , Obaru, Nagi , Kunitake, Masashi , Mizokami, Hiroshi and Hirayama, Chuichi(2003) 'Removal of DNA from a Protein Solution with Cross-Linked Poly(Ethyleneimine) Spherical Particles', Journal of Liquid Chromatography & Related Technologies, 26: 2, 231 — 246
To link to this Article: DOI: 10.1081/JLC-120017165
URL: http://dx.doi.org/10.1081/JLC-120017165

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JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 2, pp. 231–246, 2003

Removal of DNA from a Protein Solution with Cross-Linked Poly(Ethyleneimine) Spherical Particles

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ABSTRACT

To remove nucleic acids from a solution of a cell product used as a drug, water-insoluble poly(ethyleneimine) (PEI) spherical particles were prepared by suspension cross-linking with PEI and chloromethyloxirane. The PEI content of the particles was easily adjusted by changing the PEI ratio and the CMO ratio in the cross-linking. The cross-linked PEI particles, which had diameters of 44 to 105 μm and PEI contents of 50 to 90 unit mol%, were used as adsorbents. The adsorption of DNA and

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DOI: 10.1081/JLC-120017165
1082-6076 (Print); 1520-572X (Online)

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cell products to the adsorbents was determined using a batchwise method. The larger the PEI content of the adsorbent, the larger the DNA-adsorbing activity of the adsorbent. The apparent dissociation constant between the DNA (purified DNA from salmon spermary) and the adsorbent, decreased from 8.5×10^{-8} to 9.5×10^{-10} M with an increase in PEI ratio from 50 to 90 unit mol% under physiological conditions (ionic strength (μ) = 0.17, pH 7.2). On the other hand, the adsorbing activity of bovine serum albumin also increased with increasing PEI ratio of the adsorbent from 70 unit mol% or higher, but sharply decreased with increasing of the buffer. The adsorbing activity of γ-globulin increased with decreasing PEI ratio to 70 unit mol% or lower. As a result, when the cross-linked PEI particles, having a PEI ratio of 80 unit mol%, were used as the adsorbent, they only selectively removed DNA from various protein solutions at an $\mu = 0.17$ and a pH of 7.2. The particles decreased the concentration of DNA in each protein solution to less than 10 ng mL^{-1}, and the recovery rate of protein was more 97% in all cases.

Key Words: DNA removal; Cross-linked poly(ethyleneimine) particle; Pore size; Bovine serum albumin.

INTRODUCTION

In the biotechnology industry, various continuous cell lines, such as the Chinese Hamster Ovary cell line, are widely used for producing recombinant DNA products, such as protein and protective antigen. These cellular products are always contaminated with their oncogenes in the residual host cell DNA. Therefore, such contaminants have to be removed from biopharmaceutical product solutions used for intravenous administration, because of concerns about the possibility of cellular transformation by this potentially oncogenic DNA.[1] In 1997, a World Health Organization consultative group recommended that the safety risk was negligible or non-existent in products that contained less than 10 ng per dose of cellular DNA.[2]

To remove DNA from protein solutions, the selective adsorption method has proven to be most effective. In order to achieve a selective removal of DNA from solutions of high-molecular-weight compounds, such as proteins, by using adsorbents, it is necessary to consider not only the chemical and physical structures of DNA but also those of adsorbents and proteins, and the solution conditions. In physiological solutions, DNA molecules exist in a wide range of sizes, from $M_w 1 \times 10^4$ to 1×10^6, or larger. On the other hand, the molecular weights of proteins are generally about 1×10^4 to

5×10^5. Therefore, it is extremely difficult to separate DNA from protein only by size-separation methods, such as size-exclusion chromatography or ultrafiltration.

Already, it has been reported that various cationic polymer adsorbents, such as histidine-immobilized Sepharose[3] and Chitosan particles,[4,5] were very useful as adsorbents for anionic bio-related polymers, such as lipopolysaccharide (LPS) and nucleic acids (DNA and RNA). However, it has been reported[3] that these adsorbents cannot selectively remove DNA from acidic protein solutions, such as bovine serum albumin (BSA), because of their high adsorption for both DNA and BSA. We attempted, therefore, to develop DNA adsorbent capable of retaining high selectivity for DNA under physiological conditions. We have already found, that aminated poly(γ-methyl L-glutamate) (PMLG) spherical porous particles showed superior DNA selectivity than commercial DNA adsorbents based on chitosan.[6] However, a great disadvantage of this type of adsorbent is the low chemical stability of an ester bond originating from the side chain of PMLG.[7] Thus, their regeneration at high and low pH is ruled out.

In this work, we developed cross-linked poly(ethyleneimine) (PEI) spherical particles as a novel adsorbent, from PEI and chloromethyloxirane (CMO). The PEI has the characteristics of a polycation with long and flexible chains of amino-groups, and has higher pK_a (8.7) than chitosan ($pK_a = 6.2$). E. Unsal et al. reported[8] that PEI-attached poly(*p*-chloromethylstyrene) beads showed high DNA adsorption, and that they were suitable materials for the immobilization of DNA. Thus, it is expected that the cross-linked PEI particles also show high DNA-binding activity. The particles, being composed of —CHNH—bonds by the cross-linking of PEI and CMO, are also expected to keep their entire structure in solutions of high and low pH.

This paper first describes the effects of the PEI content of PEI/CMO particles on the adsorption of purified DNA or protein by the particles, and provides methods for the chromatographic removal of DNA from protein solutions using these particles.

EXPERIMENTAL

Materials

A 30 wt% PEI aqueous solution (degree of polymerization = 1600, $M_w = 70{,}000$) was purchased from Wako Pure Chemical (Osaka, Japan). CMO was purchased from Nacalai Tesque (Kyoto, Japan). The purified nucleic acids [DNA from salmon spermary ($M_w = 3 \times 10^5$) and RNA from

yeast ($M_w = 2.5 \times 10^5$)] were purchased from Wako Pure Chemical Ind. Ltd., Osaka. Egg albumin ($M_w = 4.5 \times 10^4$, pI = 4.6), bovine serum albumin (BSA) ($M_w = 6.9 \times 10^4$, pI = 4.9), myoglobin (from horse heart, $M_w = 1.8 \times 10^4$, pI = 6.8), γ-globulin (from human serum, $M_w = 1.6 \times 10^5$, pI = 7.4), cytochrome C (from horse heart, $M_w = 1.3 \times 10^4$, pI = 10.6), and lysozyme (from, $M_w = 1.5 \times 10^4$, pI = 11) were purchased from Sigma Chemical Co. Ltd. The fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride hydrate[9] for fluorometric analysis was purchased from Nacalai Tesque. The chitosan particles (Kurimuver II)[4,5] were purchased from Kurita Water Industries Ltd., Tokyo and used as a standard adsorbent.

Preparation of Adsorbents

PEI/CMO spherical particles were prepared by suspension cross-linking (Fig. 1) as follows: A 30 wt% PEI aqueous solution and cross-linking agent of CMO were mixed at 0°C. The mixture was suspended in a paraffin liquid. The suspension was stirred at 0°C for 30 min and then at 80°C for 24 h. The particles obtained were washed successively with acetone, chloroform, methanol, ethanol, and distilled water. All particles obtained with diameters from 44 to 105 μm were used as adsorbents.

Determination of Pore Size and Amino-group Contents

The pore size of the matrix in the adsorbent was estimated as the molecular mass exclusion (ML_{im}) from the calibration curves by aqueous size exclusion chromatography, as previously described.[10] The ML_{im} values were reduced as the molecular mass of polysaccharide. Homogeneous series of the polysaccharides (pullulan and maltose) were used as the permeable substances. The amino-group content of the adsorbent was quantified by pH titration and by elemental analyses.

DNA and Protein Assay

The DNA concentration in the sample solution was determined by fluorometric analysis[11] with a Spectrofluorophotometer FP-6500 (JASCO), using the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride hydrate. The protein concentration was measured at 280 nm with a Spectrophotometer UVIDEC-660 (JASCO).

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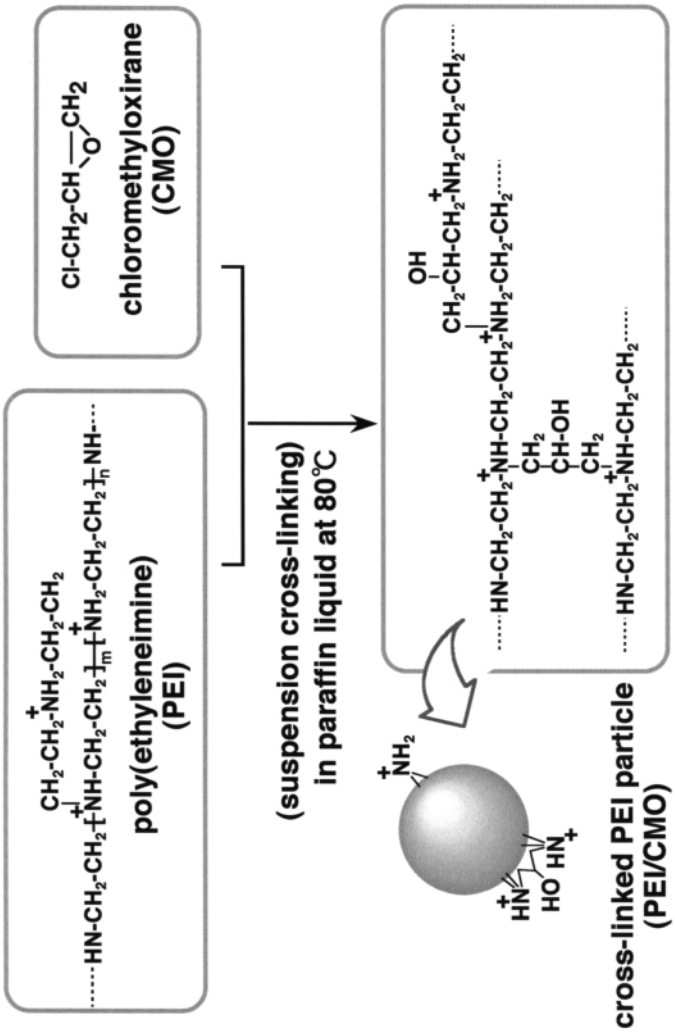

Figure 1. Preparation of cross-linked PEI/CMO spherical particles and their structure and charge distribution.

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Adsorption of DNA and Other Cellular Products

Nucleic acids (DNA or RNA) were dissolved in the following buffers: 0.02 M sodium acetate (pH 4, 5); 0.02 M phosphate (pH 6, 7, 8); 0.02 M Tris (pH 9). The ionic strength of the buffer was adjusted by changing the content of sodium chloride. The adsorption of nucleic acids was measured by a batchwise method as follows: the adsorbent was washed and equilibrated with various buffers with different pH and ionic strength (μ). A 0.1- to 0.4-g portion of wet adsorbent was suspended in 2–4 mL of DNA or RNA 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. The apparent dissociation constant ($K_{d,app}$) between the DNA and the adsorbent was estimated by adsorption isotherm, as previously described.[3,6] The adsorption of cell products other than DNA was investigated by a method similar to DNA-binding assay.

RESULTS AND DISCUSSION

Dissociation Constant Between DNA and the Adsorbent

In order to achieve the selective adsorption of DNA, it is important to decrease the interaction between the adsorbent and other cellular products, such as protein. We had already found that the protein-adsorbing activity of the aminated PMLG adsorbent increased drastically with increasing pore size in the adsorbent's matrix to sizes over the of the protein.[12] Furthermore, it was clear that the adsorbent showed good DNA-adsorbing activity, even at small pore sizes (below 10^4 as ML_{im} of polysaccharides).[6] These results suggest that adsorbents with ML_{im} below 10^4 would adsorb DNA selectively from the protein solution. Therefore, various cross-linked PEI (PEI/CMO) particles with pore sizes of ML_{im} 5×10^2 to 9×10^3 were prepared as adsorbents (Table 1). The amino-group content of the adsorbent was easily adjusted by changing the PEI ratio and the CMO ratio in cross-linking. When the unit-molar ratio of CMO (in cross-linking) increased from 10 to 50%, the amino-group content of the particles, their pore size, and their degree of swelling (in water) also decreased from 7.8 to 2.1 meq g^{-1}, from 9×10^3 to 5×10^2, and from 15.4 to 2.4 wet-mL/dry-g, respectively. We suggest that an increase in the content of the cross-linking agent lead to an increase in the hydrophobicity of the adsorbent, resulting in a decrease in its degree of swelling in water.

In the removal of DNA from a dilute DNA solution by adsorption, the DNA-removing activity depends more on the $K_{d,app}$ between DNA and the adsorbent than on the adsorbing capacity. The adsorbing activities of the adsorbents for

Table 1. Characteristics of cross-linked PEI adsorbents.

Absorbent	Molar ratio (mol%) PEI[a]	Molar ratio (mol%) CMO	Amino group content[b] (meq g^{-1})	Pore size of matrix (ML_{im})[c]	S_d[d] (wet-mL/dry-g)	Adsorbing capacity of DNA (mg mL^{-1} absorbent)	$K_{d,app}$[e] of DNA (M)
PEI/CMO = 90/10	90	10	7.8	9×10^3	15.4	5.5	9.5×10^{-10}
PEI/CMO = 80/20	80	20	7.1	2×10^3	5.8	5.4	1.8×10^{-9}
PEI/CMO = 70/30	70	30	2.9	1×10^3	3.1	3.2	3.5×10^{-9}
PEI/CMO = 60/40	60	40	2.5	1×10^3	2.7	2.9	8.5×10^{-8}
PEI/CMO = 50/50	50	50	2.1	5×10^2	2.4	2.7	9.3×10^{-8}
Chitosan particles [f]	—	—	3.6	1×10^6	16.5	17.0	9.3×10^{-9}

[a]Unit mol% of PEI in the adsorbent (1 unit = —(CH_2—CH_2—NH)—; degree of polymerization = 1600).
[b]Content of amino groups in the adsorbent.
[c]Value deduced as a molecular weight of polysaccharide.[10]
[d]Degree of swelling in water.
[e]The DNA-adsorption capacity per mL adsorbent and the apparent dissociation constant, ($K_{d,app}$) of DNA to adsorbent were estimated by adsorption isotherm, as described previously.[3,6] The adsorption isotherm of DNA was determined using a batchwise method with 0.1 mL of wet adsorbent and 4 mL of a DNA solution (DNA from salmon spermary (M_w 3×10^5): 1 to 1000 μg mL^{-1}, pH 7.2, μ = 0.17). The $K_{d,app}$ was expressed in mol L^{-1} (M) of M_w of DNA.
[f]Kurimover-II.[4,5]

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DNA were examined by a batchwise method with various kinds of buffers. The purified DNA (from salmon spermary) was used as a standard DNA sample. As shown in Table 1, while the PEI ratio increased from 50 to 90 unit mol%, the DNA-adsorbing capacity increased from 2.7 to 5.5 mg (as purified DNA from salmon spermary, $M_w = 3 \times 10^5$) per mL of wet adsorbent, and the $K_{d,app}$ of DNA to the adsorbent decreased remarkably from 9.3×10^{-8} to 9.5×10^{-10} M, under physiological conditions ($\mu = 0.17$, pH 7.2). Although chitosan particles showed the largest DNA-adsorbing capacity (17.0 mg mL^{-1}), because of the entry of the DNA into the pores (ML_{im} 1×10^6) of the particles, PEI/CMO adsorbents with PEI ratios of 90 unit mol% (PEI/CMO = 90/10) had the smallest $K_{d,app}$ values (9.5×10^{-10} M). Even in PEI/CMO = 80/20 and 70/30 adsorbents, the $K_{d,app}$ value of each adsorbent was smaller than that of the chitosan particles. The smaller the $K_{d,app}$ value, the stronger the DNA-removing activity of the adsorbent.[6] These results indicate, that the DNA-removing activity of PEI/CMO adsorbents (PEI ratio of 70 to 90 unit mol%) are higher than those of chitosan particles when the removal of DNA from a dilute DNA solution is attempted at $\mu = 0.17$ and pH 7.2.

Effects of Various Factors on Adsorption of DNA and Protein

For the selective adsorption of DNA, it is necessary to check the interaction between the adsorbent and cellular products. Figure 2 shows the effect of PEI content in the adsorbent on adsorption of cellular products (DNA from salmon spermary, BSA, and γ-globulin) at pH 7.0 and an $\mu = 0.05$. The DNA-adsorbing activity increased from 63 to 99% with an increase in PEI ratio from 50 to 80 unit mol%. The PEI/CMO = 80/20 and 90/10 adsorbents with high PEI ratio (80 and 90 unit mol%, respectively) showed excellent adsorbing activity; they were able to decrease the concentration of DNA from 500 μg/mL to less than 10 ng/mL. The adsorption of BSA was also increased from 2 to 34%, with an increase in PEI ratio from 60 to 90 unit mol%. By contrast, the adsorption of γ-globulin increased from 2% to 35, with a decrease in the PEI ratio from 80 to 50 unit mol% (with an increase in the ratio of CMO from 20 to 50 mol%). Little basic protein, such as lysozyme, was adsorbed (<2%) by any of the PEI/CMO adsorbents.

The effects of μ and pH on DNA adsorption by PEI/CMO = 80/20 and 90/10 adsorbents were compared with those of the chitosan particles (Figs. 3a, b). As shown in Fig. 3a, the higher the μ of the buffer, the lower the DNA-adsorbing activity of each adsorbent. At high $\mu = 0.6$ to 1.0, chitosan particles always showed greater DNA-adsorbing activity. For a wide pH range from 4 to 9, as shown in Fig. 4b, each PEI/CMO adsorbent showed a high DNA-adsorbing activity (82–99%). Higher DNA-adsorbing activity was

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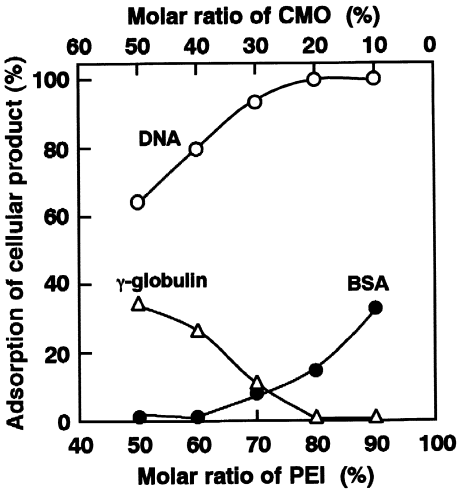

Figure 2. Effect of molar ratio of PEI in adsorbents on the adsorption of cellular products. The adsorption of a cellular product was determined by a batchwise method with 0.2 mL of wet adsorbent and 2 mL of a sample (500 $\mu g \cdot mL^{-1}$) solution (pH 7.0, $\mu = 0.05$).

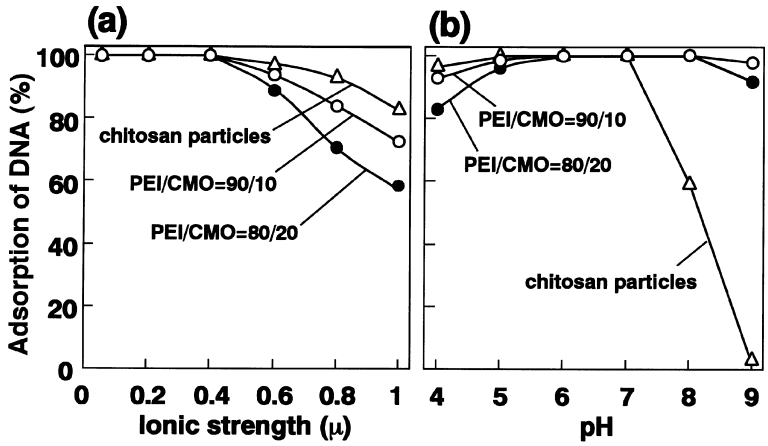

Figure 3. Effects of the buffer's ionic strength (a), and its pH (b) on the adsorption of DNA by PEI/CMO = 80/20, 90/10, and chitosan adsorbents. The adsorption of DNA was determined by a batchwise method with 0.2 mL of wet adsorbent and 2 mL of a DNA solution (purified DNA from salmon spermary: 500 $\mu g \cdot mL^{-1}$).

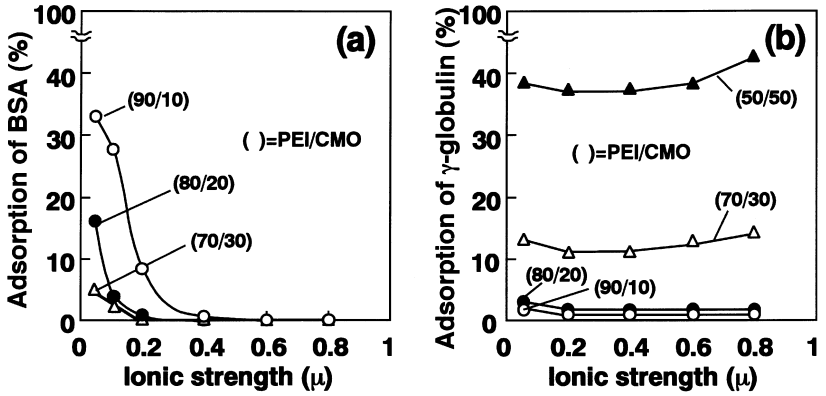

Figure 4. Effect of a buffer's ionic strength on the adsorption of (a) BSA, and (b) γ-globulin by various PEI/CMO adsorbents. The adsorption of the protein was determined using a batchwise method with 0.2 g of the wet adsorbent and 2 mL of a protein solution (500 μg/mL, pH 7.0, and ionic strength of $\mu = 0.05$–0.8).

observed in the PEI/CMO = 90/10 with the larger PEI content. On the other hand, chitosan particles showed high adsorption for DNA only in the range of pH 4 to 7. This result shows that DNA is bound more strongly by PEI/CMO than by chitosan particles at high pH (8 and 9). This difference is attributable to the fact that the pK_a of the PEI ($pK_a = 8.7$) is higher than that of chitosan ($pK_a = 6.2$). This is because the useful pH range for DNA adsorption increased with increasing pK_a of the adsorbent. As a result, it was found that PEI/CMO = 80/20 and 90/10 showed high DNA-removing activity at $\mu = 0.05$ to 0.4 and over a wide pH range of 4 to 9.

The effect of the μ of the buffer on the adsorption of BSA and γ-globulin was examined with various PEI/CMO adsorbents at $\mu = 0.05$ to 0.8 and pH 7.0 (Figs. 4a, b). The BSA-adsorbing activity of the adsorbent increased with decreasing ionic strength, as shown in Fig. 4a. At $\mu = 0.05$ to 0.2, PEI/CMO = 90/10, with the largest PEI content, always showed the highest BSA-adsorbing activity. When the ionic strength was increased to $\mu = 0.4$ or higher, the BSA adsorption was <2% in all of the adsorbents. By contrast, PEI/CMO = 50/50, with the largest CMO content, always showed the highest γ-globulin-adsorbing activity at all ionic strengths (Fig. 4b), 38 to 43% of γ-globulin adsorption was adsorbed. The adsorption by both PEI/CMO = 80/20 and 90/10 was found to be less than 2% under similar conditions.

Table 2 shows the adsorption activities of the cell products by PEI/CMO = 80/20 and 90/10 adsorbents, under the physiological conditions of pH 7.2 and $\mu = 0.17$. The binding force of each adsorbent to the DNA,

Table 2. Adsorption of cellular products by PEI/CMO adsorbents.

		Adsorption[a] (%) at *pH 7.2, $\mu = 0.17$)	
Sample	pI	PEI/CMO = 80/20	PEI/CMO = 90/10
Egg albumin	4.6	3	15
BSA	4.9	2	17
Myoglobin	6.8	<1	<1
γ-Globulin	7.4	2	2
Cytochrome C	10.5	<1	<1
Lysozyme	11.0	<1	<1
LPS (*E. coli* UKT-B)		84	91
LPS (*E. coli* O111:B4)		90	95
DNA (salmon spermary)		99	96
RNA (yeast)		94	85

[a]The adsorption of a cellular product was determined using a batchwise method with 0.2 mL of wet adsorbent and 2 mL of a sample solution (100 μg mL^{-1}, pH 7.2, μm = 0.17).

RNA, and LPS was stronger than to the protein (pI = 4.6–11.0). As for PEI/CMO = 80/20, each protein was hardly adsorbed (<3%). On the other hand, PEI/CMO = 90/10 adsorbed acidic proteins, such as egg albumin (15%) and BSA (17%).

From these results (Figs. 2–4 and Tables 1, 2), we assumed that the adsorbing-activity of PEI/CMO adsorbents for cellular products was induced by the simultaneous effects of their cationic properties and hydrophobic or other properties. Nucleic acids (DNA and RNA) are polynucleosides having anionic regions (phosphate groups), pentoses, purine bases, and pyrimidines, and, thus, the charge of DNA is anionic at pH values greater than its pK_a (<2). The charge of BSA is also anionic at pH values greater than 4.9 (its pI). The adsorption of DNA and BSA increased with increasing PEI content of the adsorbent (Fig. 2). It is also dependent on the ionic strength and pH of the buffer (Figs. 3, 4a, respectively). This suggests PEI/CMO adsorbents adsorb DNA and BSA mainly by ionic interaction. On the other hand, the ionic interaction of the adsorbent with γ-globulin (pI 7.4) is not induced at pH 7.0, since the charge of the protein is cationic at a pH under its pI value. γ-globulin is a weakly hydrophobic protein, and its adsorption by PEI/CMO adsorbent increased with an increase in hydrophobicity (content of cross-linking agent) of the adsorbent (Figs. 2, 4b). These findings suggest the participation of hydrophobic binding.

Furthermore, as shown in Table 2, the PEI/CMO adsorbents bind more strongly with DNA than protein. This is because the pK_a of the phosphate

residues of DNA is lower than the pI of protein (pI = 4.6–11.0), and probably because the DNA was adsorbed by the adsorbent, through its multipoint-attachment onto the PEI chain (a polycation) of the adsorbent surface. As a result, PEI/CMO = 80/20 can selectively adsorb DNA under physiological conditions, without the adsorption of protein.

Removal of LPS from Various Protein Solutions

For the selective adsorption of DNA, it is necessary not only to select the ligand of the adsorbent and its pK_a but also to control the conditions of the buffer (pH and μ). The effect of the ionic strength of the buffer on the selective adsorption of DNA from a BSA-containing solution was examined with the PEI/CMO = 80/20, 90/10, and chitosan adsorbents (Figs. 5a, b, and c). A BSA solution ($500\,\mu g \cdot mL^{-1}$), to which was added purified DNA ($10\,\mu g \cdot mL^{-1}$), was used as a sample solution at pH 7.0 and an ionic strength of $\mu = 0.05$ to 0.8. As shown in Fig. 5a, PEI/CMO = 80/20 selectively adsorbed DNA from a BSA solution at $\mu = 0.1$ to 0.4, without the adsorption of BSA. PEI/CMO = 90/10 was able to adsorb DNA selectively at $\mu = 0.4$ to 0.8 (Fig. 5b). The chitosan particles showed adsorbing activities for both DNA (96–99%) and BSA (9–23%) at a wide range of ionic strengths, $\mu = 0.05$ to 0.8 (Fig. 5c).

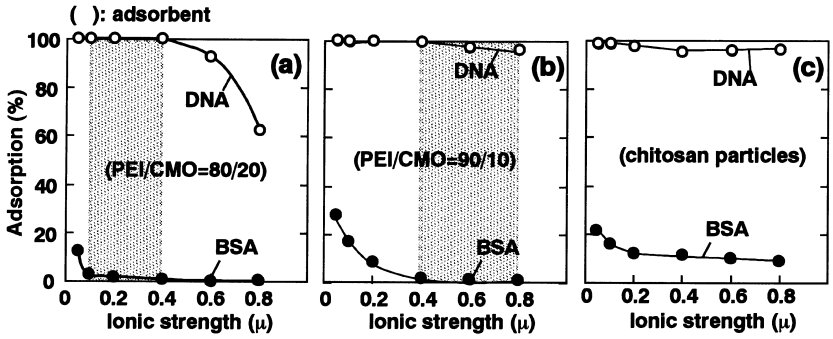

Figure 5. Effect of a buffer's ionic strength on selective adsorption of DNA from a BSA solution ($500\,\mu g \cdot mL^{-1}$, pH 7.0, and $\mu = 0.05$–0.8)) containing DNA ($10\,\mu g \cdot mL^{-1}L$) by (a) PEI/CMO = 80/20, (b) 90/10, and (c) chitosan adsorbents. The selective adsorption of DNA was determined using a batchwise method with 0.2 g of the wet adsorbent and 2 mL of a BSA-containing solution.

Table 3. Selective removal of DNA from a protein solution by PEI/CMO = 80/20 and chitosan adsorbents.

		PEI/CMO = 80/20 adsorbent		Chitosan adsorbent	
Compound	pI	Residual concentration of DNA after treatment ($ng\,mL^{-1}$)	Recidue of protein after Treatment (%)	Residual concentration of DNA after treatment ($ng\,mL^{-1}$)	Recidue of protein after treatment (%)
Egg albumin	4.6	<10	97	<10	87
BSA	4.9	<10	99	<10	88
Myoglobin	6.8	<10	99	<10	90
γ-Globulin	7.4	<10	97	<10	91
Lysozyme	11.0	<10	99	<10	82

Note: The removal of DNA was determined using a batchwise method with 0.4 mL of wet adsorbent and 4 mL of a protein solution ($1\,mg\,mL^{-1}$, pH 7.2, $\mu = 0.17$) containing DNA (from salmon spermary, $10\,\mu g\,mL^{-1}$).

The DNA-removing activity of PEI/CMO = 80/20 was compared with that of chitosan adsorbents. Various useful protein solutions (1 mg mL^{-1}), to which was added purified DNA (10 μg/mL), were used as samples under pH 7.2 and $\mu = 0.17$ conditions. The results are shown in Table 3. It is essential to eliminate DNA to concentrations less than 10 ng mL^{-1} from fluids used for intravenous injection, so as not to elicit contamination of oncogenic DNA.[2] As shown in Table 3, PEI/CMO = 80/20 was able to remove DNA from all protein solutions to a level below the critical 10 ng · mL^{-1}, and resulted in high recoveries (97–99%) of all proteins. In contrast, the chitosan adsorbent had relatively poor protein recovery (82–90%) at all proteins, although its DNA-removing activity was strong. We suggest, that the undesirable binding between protein and chitosan adsorbent probably results from the entry of protein into the large pores (ML_{im} 1×10^6) of the adsorbent's matrix.

CONCLUSION

The present results suggest that PEI/CMO = 80/20 spherical particles can remove any DNA from biologically active substances produced, not only by traditional methods but also by recombinant DNA technology. Figure 6 shows

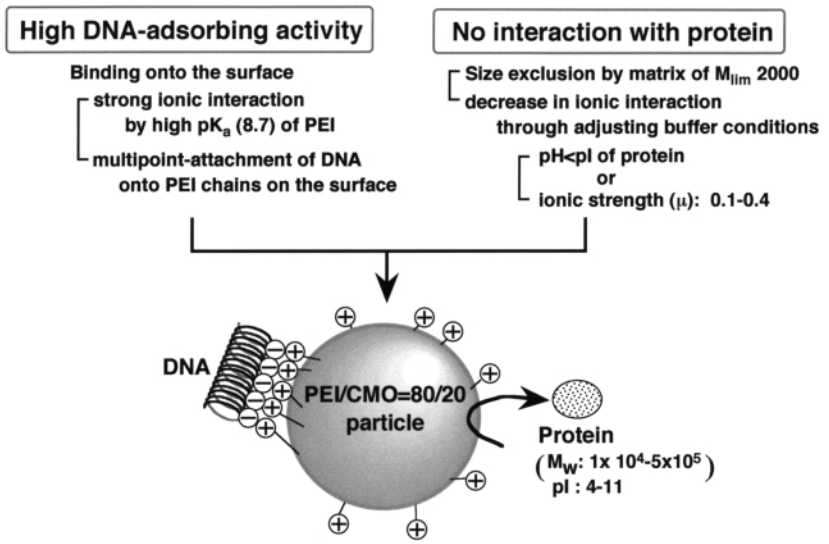

Figure 6. Several factors for the selective removal of DNA from protein solutions by PEI/CMO adsorbents.

several factors for the selective removal of DNA from protein solutions by PEI/CMO particles. The high DNA-adsorbing activity of the PEI/CMO particles is possibly due to (1) strong cationic properties by the higher pK_a of PEI (8.7) than chitosan (6.2), (2) multipoint-attachment of DNA onto the long and flexible PEI chains on the particle's surface. On the other hand, their high DNA selectivity (no interaction with proteins) is due to (1) the size-exclusion effects on protein molecules through their small pore sizes (ML_{im} 2×10^3), (2) the effect of a decrease in hydrophobic binding which arises when the content of the cross-linked agent (in the particles) is adjusted to 20 mol%, and (3) the effect of the decrease in ionic interaction which arises when the buffer's pH value or its μ is adjusted to less than the pI of the protein or to 0.1–0.4, respectively.

For practical applications, ease of regeneration is very important. The cross-linked PEI (PEI/CMO) particles can be completely regenerated by frontal chromatography with 0.2 M sodium hydroxide, followed by 2.0 M sodium chloride. Their stable structures, resisting extreme pH values, are due to their —CHNH— bonds by cross-linking. In addition, we believe that the PEI/CMO particles are better column-packing materials for DNA removing because of their higher flow-rate resistance than that of conventional polysaccharide gels.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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Received July 8, 2002
Accepted August 10, 2002
Manuscript 5902