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Pore-Size Controlled and Aminated Poly(γ-methyl L-glutamate) Particles for Selective Removal of Nucleic Acids

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ABSTRACT: This report describes a method for selective removal of nucleic acids from various protein solutions, using aminated poly(γ-methyl L-glutamate) (PMLG-NH_2) particles. The adsorbing activity for nucleic acids (purified DNA from salmon spermary) increased with increase in either the amino-group content or pore-size (molecular mass exclusion of polysaccharide, M_{lim}) of the particles. The apparent dissociation constant between the DNA and the particles (amino-group content: 3.5 meq g^{-1}) decreased from 8.2×10^{-13} mol l^{-1} (M) to 2.0×10^{-13} M with increase in pore size from 2×10^3 to 2×10^5, at pH 7.0 and an ionic strength of $\mu = 0.05$. On the other hand, the adsorbing activity of bovine serum albumin increased with increasing M_{lim} of the particles, but decreased with increasing ionic strength of the buffer. As a result, when M_{lim} was 2×10^3 and amino-group content was 3.5 meq g^{-1}, PMLG-NH_2 particles removed DNA from various DNA-containing samples, such as protein solutions, at pH 7.0 and ionic strength (μ) of 0.2. The particles also removed DNA from crude antigen solutions originating from *Bordetella bronchiseptica* and *Pasteurella multocida*. A high recovery of protective antigen (100 %) was obtained with each sample solution after removal of the DNA, and the concentration of DNA in it decreased to below 10 ng ml^{-1}.

KEY WORDS Nucleic Acid / Deoxyribonucleic Acid / Aminated Poly(γ-methyl L-glutamate) Particle / Selective Removal / Pore Size / Amino-Group Content / Bovine Serum Albumin /

In the field of recombinant DNA technology, removal of DNA from crude drug materials is very important, because of the apprehension of the nonspecific pathophysiological reactions in mammals by injection of the fluid including recombinant DNA.[1] The methods of the sucrose density gradient centrifugation[2,3] and gel filtration[4,5] have been used in attempts to purify the antigen in crude antigen solution from cell extract. However, these methods are no efficient and practical way of removing DNA from a solution that contains high-molecular-weight substances such as antigens.

For removal of nucleic acids from solutions of cell products, such as proteins, adsorption method has proved to be most effective. Recently, some adsorbents for nucleic acids, chitosan particles[6] and histidine-immobilized Sepharose,[7,8] have become commercially available. Although these adsorbents have high capacities of adsorbing DNA, adsorption of such acidic protein as bovine serum albumin (BSA) is also caused at a low ionic strength of $\mu = 0.02$ and a neutral pH value. As the ionic strength increased, the adsorption of BSA by the adsorbents decreased, but this was accompanied by a decrease in the adsorption of DNA.[8]

We attempted, therefore, to develop new adsorbents which are capable of retaining a high selectivity for nucleic acids in solutions of high-molecular weight compounds over a wide range of ionic strength. We previously reported that aminated poly(γ-methyl L-glutamate) (PMLG) particles, which have diaminoethane as a ligand, have a high adsorbing activity of endotoxin (a pyrogenic lipopolysaccharide; LPS).[9,10] LPS is an amphipathic substance having both anionic (phosphoric acid groups) and hydrophobic regions (lipophilic groups).[11] Nucleic acids are also high-molecular weight substances with phosphoric acid groups.[12] Therefore, we considered that aminated PMLG particles may be used as selective adsorbents for nucleic acids.

In this paper we consider first the effects of amino-group content and pore size of the adsorbent on the adsorption of purified DNA or various other cell products by PMLG-NH_2 particles. We then describe the method of eliminating DNA from various protein solutions using PMLG-NH_2 particles.

EXPERIMENTAL

Materials

PMLG was kindly supplied by Ajinomoto Co., Inc., Tokyo. Diaminoethane was purchased from Nacalai Tesque, Kyoto. The purified nucleic acids (DNA from salmon spermary (M_w: 3×10^5), DNA from calf thymus (M_w: 1.5×10^5), DNA from human placenta (M_w: 1×10^5), RNA from yeast (M_w: 2.5×10^5)) were purchased from Wako Pure Chemical Ind. Ltd., Osaka.

Ovalmin (from eggs, M_w: 4.5×10^4, p*I*: 4.6), albumin (from bovine serum, M_w: 6.9×10^4, p*I*: 4.9), insulin (from bovin pancreas, M_w: 3.6×10^4, p*I*: 5.3), fibrinogen (from human plasma, M_w: 3.4×10^5, p*I*: 5.5), Myoglobin (from horse heart, M_w: 1.8×10^4, p*I*: 6.8) , γ-globulin (from human serum, M_w: 1.6×10^5, p*I*: 7.4), cytochrome C (from horse heart, M_w: 1.3×10^4, p*I*: 10.6) were purchased from Sigma Chemical Co., Ltd.

The cell extracts of *B. bronchiseptica* strain S1 and *P. multocida* strain 70 were provided by Chemo-Sero Therapeutic Research Institute. The cell extracts were prepared by sonication, centifugation and filtration (0.45 μm Milipore filter) as described previously.[4,5]

The fluorescent dye 4′,6-diamidino-2-phenylindole

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dihydrochloride hydrate[13] for fluorometric analysis was purchased from Nacalai Tesque. Chitosan particles (Kurimuver II) were purchased from Kurita Water Industries Ltd., Tokyo and used as a standard adsorbent. DEAE-Cellulofine (Chisso Co., Ltd., Tokyo), DEAE-Sepharose CL-6B (Pharmacia Biotech, Sweden), and Diaion-WA-30 (Mitsubishi Chemical Co., Ltd., Tokyo) were also used as adsorbents. All other chemicals were of analytical reagent grade.

Preparation of PMLG-NH_2 Adsorbent

PMLG-NH_2 particles were prepared by suspension evaporation[14,15] following aminolysis[9] (with diaminoethane) as described. The amino-group content of the adsorbent was adjusted by controlling the reaction conditions of temperature, time and the ratio of diaminoethane to the spheres. The PMLG-NH_2 particles obtained were washed successively with water, hot water, methanol and ethanol. PMLG-NH_2 particles with diameters of 44 to 105 μm, with amino-group content of <0.1 to 3.5 meq g^{-1}, and with pore size (M_{lim} as molecular mass of polysaccharide) of 2×10^3 to 2×10^5 were used as adsorbents.

Determination of Amino-Group Contents, M_{lim}, and Porosity of Adsorbent

The amino groups were quantified by pH titration and elemental analysis as previously described.[9] The pore size (M_{lim}) of the matrix in the adsorbent was estimated from the calibration curves by aqueous size exclusion chromatography as described.[10,16] Homogeneous series of pullulan and maltose were used as permeable substances. M_{lim} values of adsorbent were reduced as the molecular mass of polysaccharide.[10]

Adsorption of Nucleic Acids or Other Substances

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, 10). 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 strengths. A 0.1- to 0.4-g portion of wet adsorbent was suspended in 2—4 ml of a 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 or RNA content of the filtrate was determined.

The apparent dissociation constant (K_d) between DNA and adsorbent was estimated by adsorption isotherm as previously described.[8]

The adsorption of cell products other than DNA was investigated by a method similar to DNA-binding assay.

DNA and Protein Assay

The DNA or RNA concentration in the sample solution was determined by a fluorometric analysis with a Spectrofluorophotometer RF-540 (Shimadzu) using the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride hydrate.[17] The protein concentration was measured at 280 nm with a Spectrophotometer UV-160 (Shimadzu).

Table I. Adsorption of DNA by various adsorbents.

Name	Adsorbent: Amino-group content	M_{lim}[a]	Porosity[b]	Adsorption of DNA[c]
	meq g^{-1}-adsorbent		%	%
PMLC-1	<0.1	2×10^3	58	5
PMLG-1-NH_2-1.7	1.7	2×10^3	65	46
PMLG-1-NH_2-3.5	3.5	2×10^3	68	82
PMLG-2	<0.1	1×10^4	67	8
PMLG-2-NH_2-1.7	1.7	1×10^4	68	66
PMLG-2-NH_2-3.5	3.5	1×10^4	70	89
PMLG-3	<0.1	5×10^4	70	22
PMLG-3-NH_2-1.7	1.7	5×10^4	72	95
PMLG-3-NH_2-3.5	3.5	5×10^4	74	99
PMLG-4-NH_2-3.5	3.5	1×10^5	79	99
PMLG-5-NH_2-3.5	3.5	2×10^5	81	99
Chitosan particles	3.8	$>2\times10^6$	—	99
DEAE-Cellulofine	0.03	7×10^3	67	23
DEAE-Sepharose CL-6B	0.15	3×10^4	62	64
Diaion WA30	4.3	4×10^3	43	16

[a] Amino-groups introduced in to the adsorbent. [b] Calculated by calibration curves of SEC in aqueous solution. [c] The adsorption of DNA was determined by a batchwise method with 0.4 g of wet adsorbent and 4 ml of a DNA solution (DNA from salmon spermary: 500 μg ml^{-1}, pH 7.0, $\mu=0.05$).

Antigen Assay

The concentration of antigens (dermonectrotic toxins (DNT)) in sonic extracts of *B. bronchiseptica* and *P. multocida* were determined in guinea pig as described previously.[4] The antigen concentration was expressed in one toxic unit (TU) ml^{-1} of DNT. TU was defined as the highest dilution inducing a necrotic lesion of ≥5 mm in diameter. The total content of protein in the sonic extract was measured by the method of Lowry *et al.*[18]

RESULTS AND DISCUSSION

Effects of Amino-Group Content and Pore Size on DNA-Adsorbing Activity

The DNA-adsorbing activities of the adsorbents were examined by a batchwise method with various kinds of buffers. The purified DNA (from salmon spermary) was used as a standard DNA sample.

Table I shows the DNA-adsorbing activities of various adsorbents (amino-group content of <0.1 to 4.3 meq g^{-1}, M_{lim} of 2×10^3 to $>2\times10^6$) at pH 7.0 and ionic strength $\mu=0.05$. The various PMLG-NH_2 particles, Chitosan particles, DEAE-Cellulofine, DEAE-Sepharose CL-6B, and Diaion-WA-30 were used as adsorbents. The adsorbing activity for DNA increased with increase in either the amino-group content or pore-size (M_{lim}) of PMLG-NH_2. PMLG-NH_2 with large pore size (amino-group content: 3.5 meq g^{-1}, M_{lim}: 5×10^4—2×10^5) showed excellent adsorbing activity (99%) of DNA. The commercially available DNA adsorbent, chitosan particles, also showed high adsorbing activity (99%). In contrast, DEAE-Cellulofine, DEAE-Sepharose CL-6B, and Diaion-WA-30, which have been widely used as ion exchangers, were unsatisfactory with respect to adsorbing activity (16—64%).

When removing DNA from a solution by adsorp-

Table II. DNA-adsorption capacity and apparent dissociation constant between adsorbent and DNA

Adsorbent			Adsorption capacity of DNA	$K_{d,app}$
Name	Amino-group content / meq g^{-1}-adsorbent	M_{lim}	mg g^{-1}-adsorbent	mol l^{-1}
PMLG-1-NH_2-1.7	1.7	2×10^3	4.0	8.5×10^{-12}
PMLG-1-NH_2-3.5	3.5	2×10^3	6.5	8.2×10^{-13}
PMLG-5-NH_2-3.5	3.5	2×10^5	14.2	2.0×10^{-13}
Chitosan particles	3.8	$>2\times10^6$	15.0	7.9×10^{-13}

The DNA-adsorption capacity per g adsorbent and the apparent dissociation constant ($K_{d,app}$) of DNA to adsorbent were estimated by adsorption isotherm, as described previously.[5] The adsorption isotherm of DNA was determined by a batchwise method with 0.1 g of wet adsorbent and 4 ml of a DNA solution (DNA from salmon spermary (M_w: 3×10^5): 1 to 10^5 μg ml^{-1}, pH 7.0, $\mu=0.05$). The $K_{d,app}$ was expressed in mol l^{-1} of molecular weight of DNA.

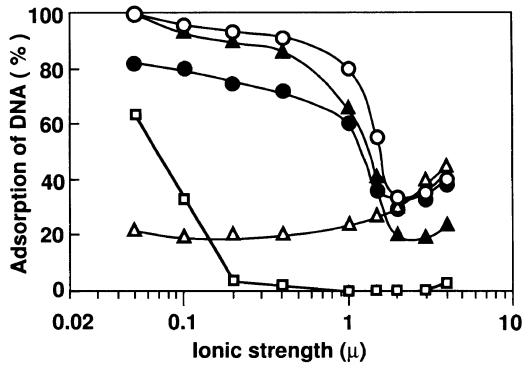

Figure 1. Effects of ionic strength on adsorption of DNA by various adsorbents. The adsorption of DNA was determined by a batchwise method with 0.2 g of the wet adsorbent and 2 ml of a DNA solution (DNA purified from salmon spermary: 500 μg ml^{-1}, pH of the buffer: 7.0). Adsorbent, pore size (M_{lim}), and amino-group content (meq g^{-1}): ○=PMLG-5-NH_2-3.5, 2×10^5, 3.5; ●=PMLG-1-NH_2-3.5, 2×10^3, 3.5; △=PMLG-5, 2×10^5, <0.1; ▲=chitosan particles (Kurimuber II), $>2\times10^6$, 3.8; □=DEAE-Sepharose CL-6B, 3×10^4, 0.15.

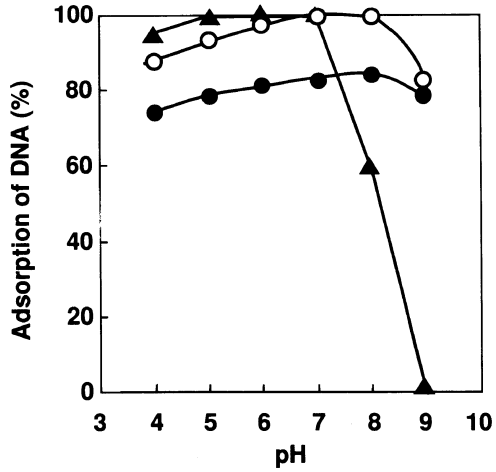

Figure 2. Effect of pH on adsorption of DNA by PMLG-5-NH_2-3.5 (○), PMLG-1-NH_2-3.5 (●), and chitosan particles (▲). The adsorption of DNA was determined by a batchwise method with 0.2 g of the wet adsorbent and 2 ml of a DNA solution (DNA from salmon spermary: 500 μg ml^{-1}, ionic strength of the buffer: $\mu=0.05$).

tion, it is necessary to check not only the adsorption capacity but also dissociation constant (K_d) between the DNA and adsorbent. The adsorption capacities and the apparent dissociation constants ($K_{d,app}$) are estimated based on adsorption isotherms as previously described.[5] The results are shown in Table II. The adsorption isotherms of DNA for PMLG-NH_2 (amino-group content: 3.5 or 1.7 meq g^{-1}, M_{lim}: 2×10^3 or 2×10^5) and the chitosan particles were determined by a batchwise method in a phosphate buffer (pH 7.0, $\mu=0.05$) by changing the concentration of DNA. The DNA-adsorbing capacity increased with an increase in the amino-group content and the pore-size. Although the chitosan particles, which have the largest pore size (M_{lim}: over 2×10^6) and amino-group content (3.8 meq g^{-1}), showed the largest DNA-adsorption capacity, the PMLG-5-NH_2-3.5 had the smallest $K_{d,app}$ value (2.0×10^{-13} M as molecular weight (3×10^5) of DNA). The smaller the $K_{d,app}$ values of the adsorbent, the higher its removing activity. These results indicate that the DNA-removing activities of the PMLG-NH_2 adsorbents (M_{lim} of 2×10^3 to 2×10^5, amino-group content of 3.5 meq g^{-1}) are higher than that of the chitosan particles when the removal of DNA from a dilute DNA solution is attempted.

Effects of Ionic Strength and pH on DNA-Adsorbing Activity

The effects of ionic strength on DNA adsorption by various adsorbents were examined in phosphate buffer (pH 7.0, $\mu=0.05$ to 4.0), and the results are shown in Figure 1. When the ionic strength was increased ($\mu=0.05$ to 2.0), the DNA-adsorbing activity of the all aminated adsorbents decreased. However, when the ionic strength was higher than 2.0, DNA-adsorbing activity of these adsorbents increased only slightly. By contrast, the adsorption of DNA to PMLG-5 (non-aminated) particles increased from 22 to 45% with increasing ionic strength from $\mu=0.05$ to 4.0. The PMLG-5-NH_2-3.5 particles showed the highest DNA-adsorbing activity (99 to 35%) at $\mu=0.05$ to 2.0. Chitosan particles also had a high DNA-adsorbing activity (99 to 20%) under these conditions. The adsorbing activity of DEAE-Sepharose CL-6B decreased markedly when the ionic strength was increased to $\mu=0.1$ or higher.

The effect of pH on DNA adsorption by PMLG-5-NH_2-3.5 and PMLG-1-NH_2-3.5 particles was compared with that of the chitosan particles. As shown in Figure 2, each PMLG-NH_2 adsorbent showed a high DNA-adsorbing activity (75—99%) at a wide pH range from 4 to 9. Higher DNA-adsorbing activity was observed in the PMLG-5-NH_2-3.5 with the larger pore size. On the

other hand, chitosan particles showed high adsorption for DNA only at pH 4 to 7.

From these results (Figures 1 and 2) we assumed that the adsorbing-activity of the PMLG-NH_2 for DNA was due to the simultaneous effects of cationic properties which originated from ligands and hydrophobic or other properties which originated from the matrix. We have previously reported that the PMLG particles show an inherent tendency towards hydrophobicity,[19] and the adsorbing-activity of PMLG particles for LPS resulted from their hydrophobic properties.[9,10] The introduction of amino groups into the PMLG particles induced an ionic interaction between LPS and the adsorbent.[9]

On the other hand, Nucleic acids (DNA and RNA) are polynucleosides having anionic regions (phosphate groups), pentoses, purine bases, and pyrimidines.[12] Their adsorbing activity, being dependent on the ionic strength and pH values of the buffer, suggests a cationic interaction. DEAE-Sepharose CL-6B adsorbs DNA mainly by cationic interaction, adsorbing less DNA at high ionic strength values (μ=0.2—4.0 and pH 7.0) (Figure 1). The adsorbing activity of the PMLG-NH_2 and the chitosan particles were also dependent on ionic strength and pH values. As shown in Figure 2, the DNA was bound more strongly by PMLG-NH_2 than by chitosan particles at a high pH (8 and 9). This difference is attributable to the fact that the pK_a of the aminated PMLG particles (pK_a: 7.9 to 8.2) is higher than that of the chitosan particles (pK_a: 6.2). Since the useful pH range for DNA adsorption increased with increase in the pK_a of the adsorbent.

Furthermore, non-aminated PMLG can adsorb DNA over a wide range of ionic strengths (μ=0.05—4.0), and non-aminated and aminated PMLG particles showed similar adsorption of DNA (35 to 45 %) at a high ionic strength of μ=4.0 (Figure 1). Matsumae *et al.*[8] reported that a hydrophobic bound was formed between cell product (endotoxin, DNA, RNA) and histidine-immobilized Sepharose under the condition of high ionic strength (μ>3). These results suggest that non-aminated and aminated PMLG particles adsorb DNA by hydrophobic effects which originate from the matrix.

Adsorption Behavior for Various Cell Products

It is important to check adsorbing activity for cell products (with the exception of nucleic acids) in order to examine selective removal of DNA. Figure 3 shows the effect of M_{lim} on adsorption of DNA, BSA, and γ-globulin by the PMLG-NH_2 particles at an ionic strength of μ=0.17 and pH 7.2, which is equivalent to that of physiological saline. The adsorption rates of all cell products increased with increasing M_{lim} of the adsorbent from 2×10^3 to 2×10^5. Only when the PMLG-NH_2 with M_{lim} of 2×10^3 and amino-group content of 3.5 meq g^{-1} (PMLG-1-NH_2-3.5) was used as the adsorbent, was the DNA adsorbed without adsorption of BSA and γ-globulin.

Figures 4a and b show the effect of ionic strength of the buffer on the adsorption of BSA and γ-globulin. The adsorption of each protein increased with increase in the M_{lim} of the PMLG-NH_2 particles. Although the PMLG-5-NH_2-3.5 (M_{lim}: 2×10^5) adsorbed BSA (97 to 8%) (Figure 4a) and γ-globulin (8 to 9%) (Figure 4b) at

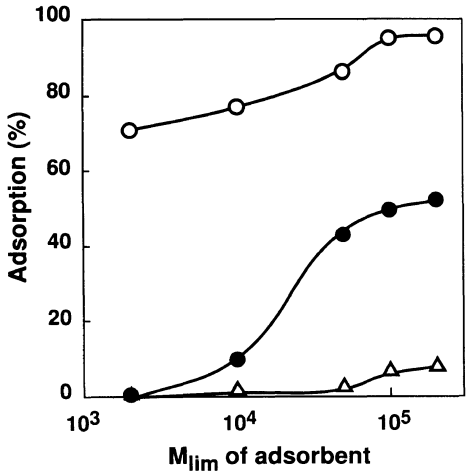

Figure 3. Effect of M_{lim} on adsorption of DNA (○), BSA (●), and γ-globulin (△) by PMLG-NH_2 adsorbents (M_{lim} 2×10^3—2×10^5, amino-group content 3.5 meq g^{-1}). The adsorption of a cell product was determined by a batchwise method with 0.4 g of the wet adsorbent and 4 ml of a cell product solution (sample: 500 μg ml^{-1}, pH 7.2, μ=0.17). M_w of cell product: DNA (salmon spermary)=3×10^5; BSA=6.9×10^4; γ-globulin=1.6×10^5.

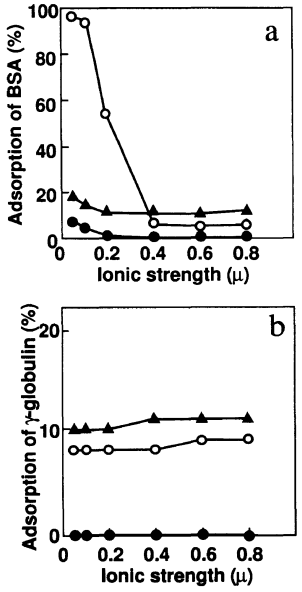

Figure 4. Effects of ionic strength on adsorption of BSA (a) and γ-globulin (b) by various adsorbents. The adsorption of a protein was determined by a batchwise method with 0.4 g of the wet adsorbent and 4 ml of a protein solution (sample: 500 μg ml^{-1}, pH of the buffer: 7.0). Adsorbent, pore size (M_{lim}), and amino-group content (meq g^{-1}): ○=PMLG-5-NH_2-3.5, 2×10^5, 3.5; ●=PMLG-1-NH_2-3.5, 2×10^3, 3.5; ▲=chitosan particles, $>2\times10^6$, 3.8.

an ionic strength of μ=0.05 to 0.8, the PMLG-1-NH_2-3.5 (M_{lim}: 2×10^3) adsorbed little protein (below 1%) under these conditions. These results (Figures 4a and b) showed that adsorption of protein caused mainly by entry of the protein into the pores of the adsorbent. This indicates that both BSA (M_w: 6.9×10^4) and γ-globulin (M_w: 1.6×10^5) can penetrate readily into the particle with M_{lim} of 2×10^5, but can not penetrate into the particle with M_{lim} of 2×10^3.

Much of the standard DNA (from salmon spermary, M_w: 3×10^5), however, was well adsorbed even by PMLG-1-NH_2-3.5 (less than the molecular mass of DNA), as shown in Figures 1, 2, and 3. The standard

Table III. Adsorption of cell product by PMLG-1-NH_2-3.5

Cell product	p*I*	Adsorption[a]/%			
		$\mu=0.05$	$\mu=0.2$	$\mu=0.4$	$\mu=0.6$
Ovalbumin	4.6	25	0	0	0
BSA	4.9	38	3	0	0
Insulin	5.3	5	1	0	0
Fibrinogen	5.5	25	2	0	0
Myoglobin	6.8	0	0	0	0
γ-Globulin	7.4	0	0	0	0
Cytochrome *c*	10.6	0	0	0	0
DNA (Salmon spermary)		99	98	90	82
DNA (Calf thymus)		99	99	93	93
DNA (Human placenta)		99	98	90	91
RNA (Yeast)		99	98	88	85

[a] The adsorption of a cell product was determined by a batchwise method with 0.4 g of wet adsorbent ($-NH_2$: 3.5 meq g^{-1}, M_{lim}: 2×10^3) and 4 ml of a sample solution (100 $\mu g\,ml^{-1}$, pH 7.0).

DNA cannot enter the pores of PMLG-1-NH_2-3.5. Therefore, we assume that the standard DNA is adsorbed on the surface of the adsorbent.

It seems that the adsorption of the protein is induced by both cationic and hydrophobic properties. Matsumae *et al.*[8] reported that acidic or neutral substances are adsorbed to histidine-immobilized Sepharose at a pH more basic than the isoelectric point of the substance under the condition of $\mu=0.02$. Aminated PMLG adsorbents has the same characteristics. The charge of BSA is anionic at pH values greater than its p*I* (4.9), and also the adsorption of BSA by the adsorbents is dependent on the ionic strength, as shown in Figure 4a. This suggests ionic interaction between the adsorbent and BSA. On the other hand, γ-globulin (p*I*: 7.4) is a neutral (p*I*: 7.4) and weakly hydrophobic protein, and its adsorption is independent of ionic strength, as shown in Figure 4b. These findings suggest the participation of hydrophobic binding.

The chitosan particles with large pore size (M_{lim} over 2×10^6) also adsorbed BSA (11—19%) (Figure 4a) and γ-globulin (10—12%) (Figure 4b) in similar conditions. Only at an ionic strength of $\mu=0.05$ to 0.2, is the BSA-adsorbing activities of PMLG-NH_2-5-3.5 higher than that of the chitosan particles although the pore size of the PMLG-NH_2 is smaller than that of the chitosan particles. This higher BSA-adsorbing activity is attributable to the fact that the pK_a of PMLG-NH_2 is higher than that of the chitosan particles, as shown in Figure 2.

Table III shows the adsorption activities of the various cell products by PMLG-1-NH_2-3.5. Myoglobin, γ-globulin (neutral proteins), and cytochrome *c* (a basic protein) were hardly adsorbed at any ionic strength value. Acidic substances were well adsorbed at pH 7.0 and $\mu=0.05$. When the ionic strength was increased ($\mu=0.2$), most acidic substances (with the exception of DNA and RNA) were scarcely adsorbed (<3%). It is suggested that the binding force of the adsorbent to the nucleic acids is stronger than that to the protein (p*I*: 4.6—10.6), because the nucleic acids have a lower pK_a than the protein.

These results (Figures 3, 4, and Table III) suggest that PMLG-NH_2-1-3.5 can adsorb DNA without affecting the protein recovery and also that proper selection for adjusting ionic strength and pH in buffer solutions enables selective removal of DNA and RNA from protein containing solutions.

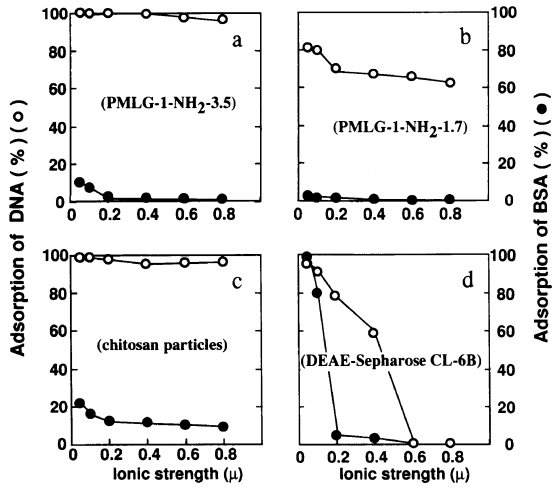

Figure 5. Effect of ionic strength on removal of DNA from a BSA solution containing DNA (BSA: 500 $\mu g\,ml^{-1}$, DNA from salmon spermary: 10 $\mu g\,ml^{-1}$, pH 7.0) by various adsorbents. The removal of DNA was determined by a batchwise method with 0.4 g of the wet adsorbent and 4 ml of a sample solution.

Table IV. Removal of DNA from various protein solutions containing DNA by PMLG-1-NH_2-3.5

Protein (p*I*)		Removal of DNA	Recovery of protein
		%	%
Ovoalbumin	(4.6)	98.6	96
BSA	(4.9)	99.4	98
Fibrinogen	(5.5)	99.9	97
γ-Globulin	(7.4)	99.9	97
Cytochrome *c*	(10.6)	99.9	99

The removal of DNA was determined by a batchwise method with 0.4 g of the wet adsorbent (amino-group content: 3.5 meq g^{-1}, M_{lim}: 2×10^3) and 4 ml of a sample solution (DNA from salmon spermary: 10 $\mu g\,ml^{-1}$, protein: 500 $\mu g\,ml^{-1}$, pH 7.0, $\mu=0.2$).

Selective Removal of DNA from Protein-Containing Solutions

The effects of ionic strength on the selective removal of DNA from a BSA-containing solution with various adsorbents were examined (Figures 5a, b, c, and d). As shown in Figure 5a, PMLG-1-NH_2-3.5 selectively removed DNA from a BSA solution at $\mu=0.2$ to 0.4. PMLG-1-NH_2-1.7 had relatively poor DNA-adsorbing activity (81—62%) at all ionic strength values although its recovery of BSA was high (<98%) (Figure 4b). The chitosan particles showed adsorbing activities for both DNA (95—99%) and BSA (9—23%) at $\mu=0.05$ to 0.8 (Figure 4c). DEAE-Sepharose CL-6B had high adsorbing activities for both at low ionic strength, $\mu=0.05$, and the adsorbing activities decreased with increase in ionic strength (Figure 4d). The chitosan particle and DEAE-Sepharose CL-6B therefore could not selectively remove DNA from a BSA-DNA solution at any ionic strengths.

Table IV shows the selective removal of DNA from various useful protein solutions containing DNA

Table V. Removal of DNA from antigen solutions originating from bacteria by PMLG-1-NH_2-3.5

Substance	Sample solution: Protein[a] / mg ml^{-1}	Sample solution: DNT[b] / TU ml^{-1}	Concentration of DNA[c]/ng ml^{-1}: Before treatment	Concentration of DNA[c]/ng ml^{-1}: After treatment	Recovery of compound after treatment/%: Protein	Recovery of compound after treatment/%: DNT
B. bronchiseptica sonicated extract	9.8	2.5×10^4	6.5×10^5	< 10	94	100
P. multocida sonicated extract	6.7	2.0×10^4	5.7×10^5	< 10	92	100

The removal of DNA was determined by a batchwise method with 0.3 ml (0.75 g) of the adsorbent and 10 ml of a sample solution in 0.02 M phosphate buffer (pH 7.2, $\mu = 0.17$). Assay methods: [a] The method of Lowry *et al.* [b] DNT assay. [c] Fluorometric analysis.

(protein: 500 μg ml^{-1}, the standard DNA: 10 μg ml^{-1}) by PMLG-1-NH_2-3.5. When the DNA adsorption was measured at pH 7.0 and $\mu = 0.2$, a high recovery of protein (96—99%) was obtained with each sample solution after removal of DNA. The concentration of DNA in each sample solution decreased from 10 to below 0.1 μg ml^{-1}.

Selective removal of DNA from crude antigen solutions from *B. bronchiseptica* and *P. multocida* was examined with PMLG-1-NH_2-3.5 particles at an ionic strength $\mu = 0.17$ and pH 7.2. The results are shown in Table V. The particles were able to remove DNA from each crude antigen solution to a level below 10 ng ml^{-1} without loss of the antigen activity.

It is essential to eliminate DNA to be at least a concentration of 10 pg ml^{-1} from drugs produced by recombinant DNA technology.[1] In this report, it was impossible to check if the DNA concentration of a sample solution after treatment was decreased to below 10 pg ml^{-1} because of the unsatisfactory sensitivity of DNA assay. However, the results (Tables IV and V) indicate that aminated PMLG particles can remove DNA from a drug to a level below 10 pg ml^{-1} without loss of proteins.

CONCLUSIONS

The present results suggest that the adsorption technique, which uses the aminated PMLG particles, can remove DNA from drugs and fluids for injection. This process did not affect the recovery of important compounds such as antigen. The high DNA selectivity of the particles is due to (1) the simultaneous effects of cationic properties of ligands and hydrophobic or other properties of matrix, (2) the exempting effects on protein molecules when the M_{lim} rate is adjusted to 2×10^3, and (3) a higher pK_a (8.2) than for Chitosan particles (pK_a: 6.0).

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