Chromatographic separation of DNA from protein solution by cellulose beads grafted with cationic polymer chains through ATRP

（ATRP 法によりカチオン性ポリマーをグラフト化させたセルロースビーズの調製とタンパク質水溶液からのＤＮＡのクロマト分離への応用）

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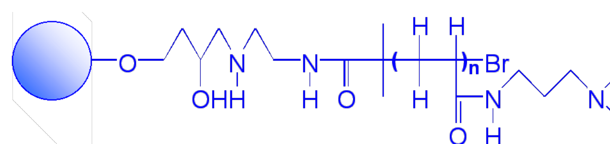
As a novel DNA adsorbent, micro-porous cellulose beads grafted with poly(dimethylaminopropylacrylamide) (polyDAPA) have been prepared by surface initiated atom transfer radical polymerization (SI-ATRP). In this paper, we report the synthesis of the DNA adsorbent by SI-ATRP, and then provide a method for the chromatographic separation of DNA from a protein solution using the beads-packed columns.



PolyDAPA-grafted cellulose beads

To synthesize the DNA adsorbent, at first, ethylenediamine was grafted onto chloromethyloxirane-activated cellulose beads. Then 2-bromo-2methylpropionyl bromide (BMPB) was introduced into the aminated beads as initiating sites for SI-ATRP.[1] Cu(I)Br/phenanthrolin catalyst-ligand system was used for DAPA polymerization in H_2O at 100°C. The content of initiating sites and the degree of polymerization (amino-exchange capacity; AEC) were adjusted by changing BMPB content of beads and polymerization time, respectively. The resulting various cationic beads, which had diameters of 44 - 105 μm and matrix's pore-sizes of 1×10^3-2×10^3 as molecular mass exclusions (M_{lim}), were used as adsorbents. We tried to separate DNA and bovine serum albumin (BSA) using cationic polymer beads packed column by chromatographic separation. DNA concentration in a sample solution was determined by fluorometric analysis using a Spectrofluorophotometer FP-6500 (JASCO, Japan) with a fluorescent dye 4',6-diamidino-2-phenylindole dihydrochloride hydrate. Protein concentration was measured using the bicinchoninic acid protein assay at 562 nm.

Chromatographic separation of BSA and DNA (salmon spermary, Mw: 3×10^5) was investigated using various polyDAPA-grafted cellulose beads packed columns (polyDAPA-cell) (Table1). It was proved that each bead selectively adsorbed DNA from the BSA/DNA solution. The polyDAPA-cell-3, which has the largest content of initiating sites (BMPB: 0.36 m-mol/g) and the largest DAPA-polymerization degree (AEC: 14.9 m-mol/g) showed the highest DNA-selectivity at pH 7.0 and ionic strength (μ) 0.2: DNA recovery was <0.1 % (<10 ng/mL) and BSA recovery was 94%. When each DNA-saturated column was then treated with the salt gradient concentration, the purified DNA was eluted successfully (DNA recovery: 13-36%). The polyDAPA-cell-1, which has the smallest content of initiating sites (BMPB: 0.21 m-mol/g) and the smallest DAPA-polymerization degree (AEC: 0.7 m-mol/g) showed the highest DNA-recovery under a salt gradient condition (0.2-2.0 M). The results indicate that polyDAPA-grafted cellulose beads allows specific separation of DNA from proteins. We assume that the remaining DNA, which was not eluted, was strongly adsorbed onto the their polyDAPA chain by hydrophobic binding and multipoint attachments.

Table 1. Chromatographic separation of BSA and DNA

Cationic beads	Content of BMPB (m-mol/g)	AEC (m-mol/g)	BSA elution buffer Ionic strength μ=0.2: Recovery of DNA %	Recovery of BSA %	DNA elution buffer 0.2-2.0M NaCl aq.: Recovery of DNA %	Recovery of BSA %
polyDAPA-cell-1	0.21	0.7	<0.1	90	36	7
polyDAPA-cell-2	0.26	1.9	2	71	15	20
polyDAPA-cell-3	0.36	14.9	<0.1	94	13	1.6

A 60 mL of BSA solution supplemented with DNA (BSA:1 mg /mL, DNA: 10 μg/mL, pH 7.0, μ = 0.2) was passed through a 3.2 mL-column at 0.2 mL/min. The DNA-saturated column was washed with 60 mL buffer (pH 7.0, μ = 0.2), and then treated with 60 mL of 0.2-2.0 M-NaCl solution (gradient condtion).

1)G. Morandi, L. Heath, and W. Thielemans,, *Langmiur*. **25**, 8280 (2009).

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