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Short communication

Rapid scaleable chromatographic purification of nucleic acids from proteinaceous mixtures

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Abstract

The present studies describe a unique HPLC method for the purification of nucleic acids from proteins, scaleable to a preparative or process level, using a Zorbax PRO-10/DN 300 Å silica packing. Columns of this material have an extremely high affinity for proteinaceous materials, and a very low affinity for nucleic acids. We demonstrate this by separating salmon DNA from bovine serum albumin; and describe use of the PRO-10/D-N column to separate plasmid DNA from bacterial lysates. The method is quick and, unlike phenol–chloroform extraction, utilizes physiological buffers to minimize hazardous operations. This LC method is faster than classical non-LC methods, is less labor intensive, and uses no organic solvents, thereby eliminating the need for post-separation recovery operations. The high recovery of DNA purified by Zorbax PRO-10/D-N provides DNA material suitable for many common molecular manipulations including restriction enzyme cleavage, ligation, and amplification.

1. Introduction

Nucleic acids for cloning purposes and for use in a variety of hybridization-based diagnostic assays generally must be highly purified and free of a broad range of impurities such as proteins and organic solvents. Traditionally, the removal of proteins from nucleic acids has been achieved by phenol–chloroform extraction [1]. The toxicity of these organic solvents requires that specialized and costly handling and disposal procedures be used [2]. An alternative method for separating proteins from nucleic acids, the McCormick process [3], involves contacting the protein–nucleic acid mixture with siliceous-based particulates that have been specially prepared to enhance their affinity for proteins and substantially decrease their affinity for nucleic acids. This solid-phase extraction protocol is characterized by single use of the silica product. Scale-up of either of these extraction procedures to produce larger quantities of a given nucleic acid is, at the very least, impractical. Saburov and co-workers [4,5] suggested the use of fluorinated polymers for the separation of nucleic acids from proteins using reversed-phase chromatography. They describe separation by chromatographic sorbent on the basis of controlled pore glasses and polytetrafluoroethylene.

We have developed an HPLC method for separation of proteins from nucleic acids by utilizing a Zorbax PRO-10/D-N column with packing prepared using the McCormick process. With this method, polyanions such as nucleic

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acids will pass through the column in a controlled fashion, to be collected at the end of the packed column, while the protein from the original mix becomes bound to the silica surface. The procedure is rapid, requiring only 15–20 min to complete; and we have demonstrated that the column may be re-used at least 200 times before regeneration is required. This chromatographic purification procedure has advantages over other techniques, particularly at the preparative or process level. We have demonstrated the utility of a commercial Zorbax PRO-10/D-N column by using it to purify (1) salmon DNA from bovine serum albumin and (2) plasmid DNA from bacterial lysates.

2. Experimental

2.1. D-N Chromatography

Zorbax PRO-10/D-N 300 Å packing material consists of uniform 10-μm spherical particles of totally porous silica. Proprietary steps yield a particle with a low surface concentration of polyvalent cations, and an enhanced concentration of mildly acidic hydroxyl groups. A standard HPLC column (25 cm × 4.6 mm I.D.) packed with PRO-10/D-N (BTR-Separations) was used for chromatography. The column was run at ambient temperature using a Rainin HPLC method manager with a Dynamax SD-1 delivery system, a Dynamax UV-1 detector, and a Macintosh LC system (Rainin, Richfield, NJ, USA). Mobile phase was 5 m*M* 2-(N-morpholino)ethanesulfonic acid (MES, Sigma, St. Louis, MO, USA), injection volume was 50 μl, and flow-rate was 0.4 ml/min.

2.2. Preparation of bacterial lysates

Escherichia coli strain XL1-Blue [6] harboring the plasmid pUC18 [7] was grown in LB media [1] supplemented with 50 μg/ml ampicillin (Sigma) with vigorous shaking at 37°C until saturation (14–20 h). Bacterial cells were harvested by centrifugation, alkaline-lysed, and neutralized as described [1] resulting in a cleared lysate consisting primarily of plasmid DNA and soluble protein.

2.3. Purification of plasmid DNA by organic extraction

Plasmid DNA was purified from cleared lysates by phenol and chloroform extraction, followed by ethanol precipitation as described [1].

2.4. Transformation assay

E. coli strain XL1-Blue was transformed by the calcium chloride procedure as described [1] and selected on LB plates supplemented with 50 μg/ml ampicillin.

2.5. Restriction and ligation analysis

Restriction enzymes and DNA ligase were purchased from New England Biolabs. (Beverly, MA, USA) and used according to the manufacturer's recommendations.

2.6. Polymerase chain reaction (PCR) analysis

A PCR kit (Perkin-Elmer Cetus, Norwalk, CT, USA) was used according to the manufacturer's recommendations. The 5′ primer is a synthetic oligomer with the sequence 5′-GCCGATATCCACCCAGAAACGCTGGTG-AA-3′, and the 3′ primer is a synthetic oligomer with the sequence 5′-CGCGATATCCCAA-TGCTTAATTCAGTGAG-3′. Thermal cycling parameters were 1′ at 94°C, 1′ at 55°C, 1′ at 72°C, 40 cycles.

2.7. Miscellaneous

Bovine serum albumin (BSA), salmon DNA, and MES were obtained from Sigma. Water was used after passing through a Milli-Q purification system (Millipore, Bedford, MA, USA).

3. Results and discussion

3.1. Separation of salmon DNA from BSA by D-N chromatography

Solutions of salmon DNA and BSA were used to optimize mobile phase and pH conditions, and to characterize chromatographic performance of the D-N HPLC column. The mobile phase of choice was found to be MES. The desired results could not be achieved with the use of a variety of phosphate buffers. Salmon DNA and BSA solutions were prepared in MES buffers ranging in pH from 4.3 to 7.1 and analyzed by D-N chromatography. The optimal pH for BSA removal (adsorbtion to the column) and minimal loss of DNA occurred at pH 7.0 (data not shown). The pH of the mobile phase was determined to be between 5.0 and 5.4. Effective separation of DNA from BSA under these conditions was demonstrated by passing the following three samples through a D-N column: (1) salmon DNA at 0.02 mg/ml in MES buffer pH 7.0, (2) BSA at 2.6 mg/ml in MES buffer pH 7.0 and (3) a mixture of salmon DNA at 0.02 mg/ml and BSA at 2.6 mg/ml in MES buffer pH 7.0. The chromatograms obtained indicate high recovery and high purification of salmon DNA from BSA using this procedure (Fig. 1).

Calculations by others indicate that an analytical column (25 cm × 0.46 cm) has a capacity of approximately 40 mg of protein, whereas a preparative column (25 cm × 1 in.; 1 in. = 2.54 cm) has a capacity of approximately 1.0 g of

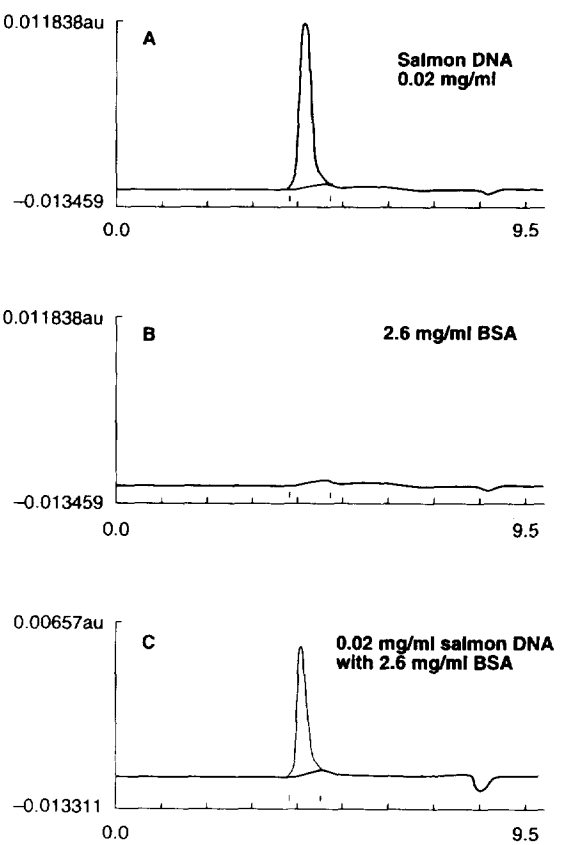

Fig. 1. Separation of salmon DNA from BSA by D-N chromatography. HPLC chromatograms (A_{260}) obtained from Zorbax PRO-10/D-N columns loaded with (A) 50 μl of salmon DNA at 0.02 mg/ml in MES buffer pH 7.0, (B) 50 μl of BSA at 2.6 mg/ml in MES buffer pH 7.0 and (C) 50 μl of a mixture of salmon DNA at 0.02 mg/ml and BSA at 2.6 mg/ml in MES buffer pH 7.0. Protein is adsorbed to the column. Time scale on the *x*-axis in minutes.

protein [8]. Thus 50-μl injections of BSA at 2.6 mg/ml suggests that the analytical column may be re-used approximately 300 times before regeneration is required. A column may be regenerated by contact with acetonitrile for 2–3 h followed by 0.1 % trifluoracetic acid for 2 h and 5 m*M* MES for 1 h.

3.2. Separation of plasmid DNA from bacterial lysate by D-N chromatography

A cleared bacterial lysate was prepared from a saturated culture of *E. coli* harboring the plasmid pUC18 as described (Experimental). The lysate was divided equally and plasmid DNA was recovered from one half by phenol and chloroform extraction, and from the other half by D-N chromatography. Recovery of plasmid DNA was determined by transformation of competent *E. coli* and selection on ampicillin plates. The elution profile of plasmid DNA from the D-N column coincides with a major peak in the chromatogram which is well separated from peaks corresponding to other lysate or buffer components (Fig. 2). Fractions (minutes) 5–7 were pooled and the yield of plasmid DNA was compared to that obtained by organic extraction. After adjusting for different final volumes, the relative recovery of plasmid DNA from D-N separation was determined to be statistically equivalent to that from organic extraction (Fig. 3). No attempt was made to determine the fate of RNA present in the crude lysate.

3.3. Molecular manipulation of plasmid DNA purified by D-N chromatography

Restriction cleavage and re-ligation tests were performed as general functional assays for plasmid DNA purified from bacterial lysates (Table 1). Plasmid DNA samples prepared by organic extraction or by D-N chromatography were subjected to cleavage by the restriction enzymes HindIII or KpnI. Each of these enzymes has one recognition site in the plasmid pUC18, and cleavage results in a linear DNA molecule which is incapable of transforming competent *E. coli* cells. Results indicate that plasmid DNA prepared by D-N chromatography is efficiently cleaved by restrictions enzymes.

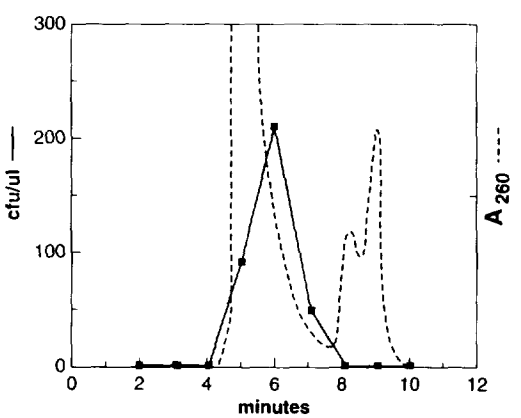

Fig. 2. Elution profile of plasmid DNA from D-N column. Cleared lysate (50 μl) prepared from *E. coli* harboring pUC18 was loaded onto a Zorbax PRO-10/D-N column and fractions collected were analyzed for plasmid DNA by transformation assay (solid line). The corresponding A_{260} HPLC chromatogram tracing (dashed line) indicates various peaks representing plasmid DNA (minutes 4–7), and non-plasmid nucleic acids or other lysate components (minutes 8–10). cfu/ul = colony-forming units per μl of column fraction.

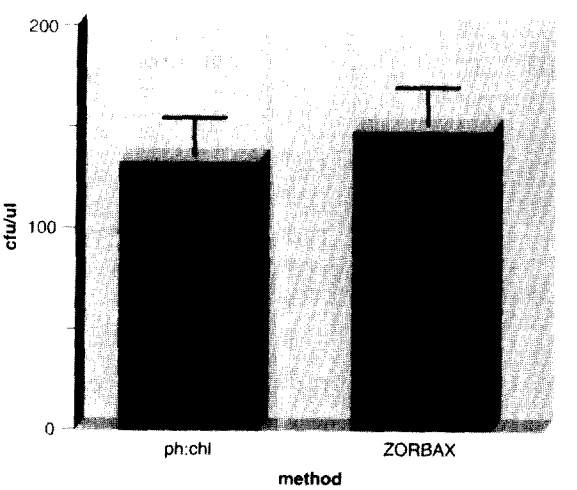

Fig. 3. Recovery of plasmid DNA from cleared bacterial lysates. Yield of plasmid pUC18 from *E. coli* lysate by Zorbax PRO-10/D-N column is compared to yield by phenol–chloroform extraction (ph:chl), as determined by transformation assay. cfu/ul = Colony-forming units per μl of final sample, after adjusting volumes for the two different methods.

Table 1
Restriction digest and re-ligation analysis of plasmid pUC18 prepared by phenol–chloroform (ph:chl) extraction and Zorbax-PRO/D-N methods

Treatment	Colony-forming units	
	ph:chl	Zorbax
None	569	701
HindIII restriction[a]	6 (99%)	13 (98%)
Re-ligation[b]	245 (43%)	287 (41%)
KpnI restriction	1 (100%)	0 (100%)
Re-ligation	245 (43%)	295 (42%)

[a] Efficiency of digestion is shown in parentheses.
[b] Efficiency of re-ligation is shown in parentheses.

Restricted plasmid DNA was also subjected to re-ligation by DNA ligase, resulting in circular plasmids which are capable of transforming competent *E. coli* cells. The termini of HindIII cleaved DNA have 5′ overhangs, while the termini of KpnI cleaved DNA have 3′ overhangs. In both cases a re-ligation efficiency of approximately 40% was obtained, which is equivalent to that obtained from re-ligated plasmid DNA prepared by organic extraction.

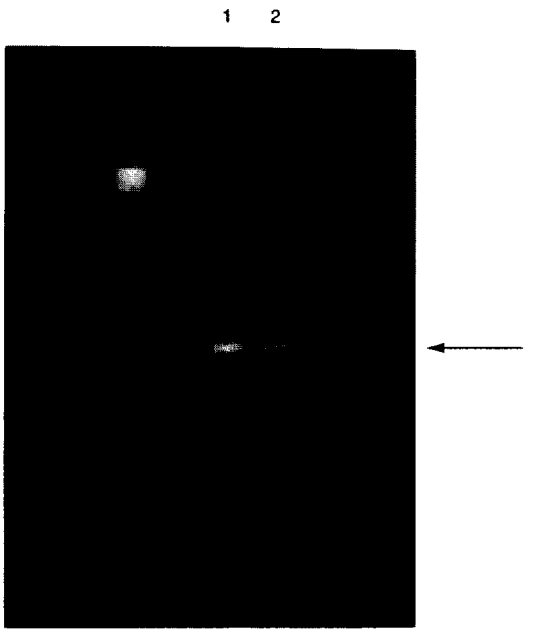

Fig. 4. Amplification of plasmid DNA. Ethidium bromide agarose gel electrophoresis of PCR amplification of the β-lactamase gene from pUC18 prepared by D-N column chromatography (lane 1) or by phenol–chloroform extraction (lane 2). Arrow indicates expected PCR product. Molecular mass size markers are at the left.

Amplification by PCR was also performed as a general functional assay for plasmid DNA purified from bacterial lysates (Fig. 4). The β-lactamase gene of the plasmid pUC18 was used as the target for PCR, and plasmid DNA samples prepared by organic extraction or by D-N chromatography were the starting material. Results indicate that plasmid DNA prepared by D-N chromatography is efficiently amplified by PCR.

4. Conclusions

We have described an HPLC method for the separation of nucleic acids from proteins based on Zorbax PRO-10/D-N silica. The process is simple, quick, effective, and uses only aqueous buffers. We demonstrated that plasmid DNA purified by this method from a complex mixture of proteins (bacterial lysates) is of equivalent yield and quality compared to preparation by organic extraction. In addition, the method is easily scaleable to a preparative or process level. The method described here could find application for the purification of nucleic acids from a variety of sources, including bacteria, plants, animals and environmental samples.

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