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Recent developments of magnetic beads for use in nucleic acid purification

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Abstract

The performance of Magarose®, an agarose-based bead containing a paramagnetic component has been evaluated. The anion exchanger DEAE-Magarose is effective at binding DNA from a crude cell lysate. The plasmid pBluescript was isolated from 1.5 ml *Escherichia coli* JM109 cell culture, following alkaline lysis yielding 8.2 μg high-quality DNA. Under similar binding conditions 21 μg of salmon sperm DNA bound to the ion exchangers. The affinity medium oligo-dT Magarose was demonstrated to bind 75 μmol of an oligo-dA probe/g of medium by hybridization. Under similar conditions mRNA could be isolated from a preparation of baby hamster cell total RNA. The magnetic susceptibility of Magarose is very high, facilitating the use of this separation technique for rapid batch chromatographic processes.

Keywords: Magnetic sorbents; Agarose beads; Adsorbents; DNA; RNA

1. Introduction

Low-pressure chromatography is routinely used in the purification of biopolymers at laboratory scale through to process scale. Low-pressure media used for adsorption chromatography are traditionally based on polysaccharide supports including agarose, cellulose and dextran [1,2]. More recently composite polymers have been introduced as the base matrix [3]. For many years, biochemists have developed purification protocols for proteins of interest utilising chromatographic techniques including ion-exchange, affinity, hydrophobic interaction and size-exclusion.

When establishing purification protocols, the protein chromatographer is faced with a myriad of related adsorbents available from different vendors each bearing similar functional groups but attached to different matrices using proprietary chemical techniques. In the field of affinity chromatography there have been several small studies comparing characteristics of the base matrix and how it may affect the affinity process [4–7]. Similarly, we have screened 70 different ion-exchange chromatography media and reported that they all perform differently under similar conditions [8] and at different scales of operation [9].

Nucleic acids provide the templates coding for the proteins associated with all cellular functions. DNA is a polymer of deoxyribonucleotides and RNA is a polymer of ribonucleotides [10]. DNA and RNA are anions at neutral pH and can therefore be isolated by anion-exchange chromatography. The DEAE-cellu-

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Table 1
Typical properties of Magarose

Property	Value	
	DEAE	Oligo-dT
Shape	Spherical	Spherical
Particle size (μm)	20–150	20–50
Nitrogen content (%, w/w)	0.3	ND[a]
Response to magnet	Rapid	Rapid

[a] ND: Not Determined.

lose paper Whatman DE81 has been reported to bind DNA fragments [11] and tRNA [12]. Plasmid DNA can be isolated from a cell lysate using adsorption chromatography to a DEAE-cellulose [13]. mRNA may be isolated from total RNA by affinity chromatography using an immobilized oligo-dT ligand [14], which selectively interacts with the poly-A tail of mRNA [15]. Under chaotropic conditions nucleic acids interact with the surface of silica [16,17] and this interaction has been exploited in many commercial purification kits.

Purified DNA can be used for a variety of purposes including polymerase chain reaction (PCR) [18], sequencing [19] and for the emerging opportunities in gene therapy [20].

In the present study we report the development of Magarose, a new agarose-based bead containing a paramagnetic component. Magarose beads may be derivatized with DEAE or oligo-dT groups for isolation of DNA or mRNA, respectively. Typical properties of Magarose are summarized in Table 1. In this study we have assessed the suitability of a magnetically driven separation process utilising Magarose beads, for the isolation of plasmid DNA from a cell lysate and mRNA from a preparation of total RNA.

2. Experimental

2.1. Materials

DEAE-Magarose and oligo-dT Magarose were obtained from Whatman (Maidstone, UK). Magnetic Beads Plasmid Mini Kit and Magnetic Beads mRNA Kit were obtained from Biometra (Göttingen, Germany). Fluorescein-labelled oligo-dA_{15} (F-dA_{15}) was obtained from Cruachem (Glasgow, UK). Salmon sperm DNA and ribonuclease A were obtained from Sigma (Poole, UK). All other chemicals were of molecular biology grade.

2.2. Salmon sperm DNA binding

A 1% (w/v) aqueous solution of salmon sperm DNA was sheared by repetitive passage through a 17 gauge hypodermic syringe needle, until the absorbance at 260 nm of a 0.5% (w/v) aqueous solution of sheared DNA was 1.00 ± 0.05, equivalent to a DNA concentration of 50 μg/ml.

A 4% (w/v) suspension of DEAE-Magarose in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.1 *M* NaCl, 0.001 *M* EDTA and 1% (w/v) Triton X-100 (STET buffer; 100 μl) was placed in a 1.5 ml microcentrifuge tube and the beads immobilized using a Magnetic Separator Stand (Biometra). The supernatant was removed and the beads washed with water (1 ml). The supernatant was removed and the beads resuspended in 50 μg/ml sheared salmon sperm DNA solution (1 ml). The contents were mixed gently for 5 min. The beads were immobilized in the Magnetic Separator Stand and the absorbance of the supernatant measured at 260 nm against a water blank.

2.3. Plasmid DNA binding

Escherichia coli JM109 cells expressing the plasmid pBluescript were grown to late log phase in Luria-Bertani broth containing 100 μg/ml ampicillin. The bacterial cells were harvested from 1.5 ml of cell culture by centrifugation at 10 000 *g* for 1 min. The pellet was resuspended in 0.05 *M* Tris–HCl buffer (pH 8.0) containing 0.01 *M* EDTA and 400 μg/ml ribonuclease A (100 μl). Cell lysis was performed by gently mixing the resuspended cell pellet with 0.2 *M* NaOH containing 1% (w/v) sodium dodecyl sulfate (SDS) (200 μl) and placing the mixture on ice for 5 min. Genomic DNA and other contaminants were precipitated by addition of 3 *M* potassium acetate (pH 5.5) previously cooled to 4°C (150 μl). The mixture was stood on ice for 10 min, and then centrifuged at 10 000 *g* for 5 min to sediment the precipitated protein, cell debris and denatured chromosomal DNA. The supernatant was

placed in a 1.5 ml DNase/RNase free microcentrifuge tube (Sorenson Biosciences, Salt Lake City, UT, USA) and a 4% (w/v) suspension of DEAE-Magarose in STET buffer (150 μl) added. The suspension was gently mixed for 5 min at room temperature. The beads were immobilized using a Magnetic Separator Stand and the supernatant removed. The beads were washed by resuspension in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.4 *M* NaCl and 0.001 *M* EDTA (400 μl). After immobilization of the beads, the supernatant was discarded and the plasmid DNA desorbed by addition of 0.01 *M* Tris–HCl buffer (pH 8.0) containing 1 *M* NaCl and 0.001 *M* EDTA (200 μl). The beads were immobilized and the supernatant transferred to a fresh DNase/RNase free microcentrifuge tube. Absolute ethanol (500 μl) pre-cooled to −20°C and 7.5 *M* ammonium acetate (20 μl) were added to the supernatant containing eluted plasmid DNA (200 μl) and the mixture stored at −20°C for 10 min. The precipitated DNA was collected by centrifugation at 15 000 *g* for 30 min at 4°C and the supernatant discarded. The pellet was washed with 70% (w/v) cold ethanol (50 μl) and centrifuged at 15 000 *g* for 10 min at 4°C. The supernatant was discarded and the pellet allowed to air-dry at room temperature for 10 min. The pellet was re-dissolved in 0.01 *M* Tris–HCl buffer (pH 8.0) containing 0.001 *M* EDTA (50 μl). The DNA solution was assayed by absorption measurement at 260 nm and 280 nm and by agarose gel electrophoresis.

2.4. Fluorescein-labelled oligo-dA binding

0.375 μM F-dA_{15} in 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.5 *M* NaCl, 0.001 *M* EDTA and 0.1% (w/v) Tween-20 (50 μl) was incubated with 1% (w/v) oligo-dT Magarose in phosphate-buffered saline (PBS) (pH 7.4) containing 0.02% (w/v) NaN_3 (10 μl). The suspension was incubated at room temperature for 5 min. The beads were immobilized in a Magnetic Separator Stand and the supernatant was discarded. The beads were washed with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.15 *M* NaCl and 0.001 *M* EDTA (50 μl). The beads were immobilized and the supernatant discarded. The wash step was repeated. Bound F-dA_{15} was desorbed by incubation with 0.002 *M* EDTA (50 μl) at 65°C for 5 min. The beads were immobilized, the supernatant collected and assayed for F-dA_{15} content by fluorimetry using an excitation wavelength of 496 nm and emission at 520 nm in a Dynatech Fluorolite model 1000 microtitre plate fluorimeter (Dynatech, Chantilly, USA).

2.5. mRNA isolation

2.6 mg of total RNA was isolated from baby hamster cells using the Qiagen RNeasy kit (Qiagen, Hilden, Germany).

1% (w/v) oligo-dT Magarose in PBS (pH 7.4) containing 0.02% (w/v) NaN_3 (100 μl) were transferred to a DNase/RNase free microcentrifuge tube and immobilized in a Magnetic Separator Stand. The supernatant was discarded and the beads resuspended in 0.02 *M* Tris–HCl buffer (pH 7.5) containing 1 *M* NaCl and 0.002 *M* EDTA (100 μl). The beads were immobilized and the supernatant discarded. 100 μl of the total RNA solution (168 μg) was heated to 65°C for 2 min, and then diluted with 0.02 *M* Tris–HCl buffer (pH 7.5) containing 1 *M* NaCl and 0.002 *M* EDTA (100 μl). The RNA solution was added to the pre-equilibrated oligo-dT Magarose and gently mixed at room temperature for 5 min. The beads were immobilized and the supernatant discarded. The beads were washed using 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.15 *M* NaCl and 0.001 *M* EDTA (150 μl). The beads were immobilized and the supernatant discarded. The wash procedure was repeated. mRNA was desorbed by incubation of the beads with 0.002 *M* EDTA (80 μl) at 65°C for 2 min. The beads were immobilized and the supernatant transferred to a fresh DNase/RNase free tube. The supernatant (10 μl) was assayed for the presence of mRNA qualitatively by agarose gel electrophoresis.

3. Results and discussion

Magnetic field based separations are gaining importance in chromatography [14,21,22]. The paramagnetic component of a chromatographic support facilitates rapid immobilization in a batch chromatographic step. The Magarose beads described in this paper respond very rapidly to a magnetic field,

typically being immobilized from a suspension in less than 10 s, leaving a clear supernatant which can readily be removed by aspiration or decantation. This enables the chromatographic protocols to be carried out rapidly, with minimal mobile phase carry-over from step-to-step resulting in short process time, economic use of reagents and most importantly a decreased likelihood of product contamination. Typical test data on the Magarose products are summarized in Table 1. The beads are not monodispersed but due to their rapid response to a magnetic field and the fact that our target separations are simple batch techniques with step elution, this is not considered an issue. The protocols used in bead manufacture render them physically and chemically robust and they are stable to heating at up to 100°C for several minutes with no loss of shape or apparent performance. The DNA binding properties of DEAE-Magarose are summarized in Table 2. The data demonstrate that DEAE-Magarose binds at least 14 μg of salmon sperm DNA per 100 μl of 4% (w/v) suspension. In a plasmid DNA isolation following an alkaline lysis procedure, DEAE-Magarose [4% (w/v) suspension, 150 μl] isolated approximately 8.2 μg of pBluescript DNA from 1.5 ml *Escherichia coli* JM109 cell culture (Table 2). As indicated by the A_{260}/A_{280} ratio the DNA was of high purity. Agarose gel electrophoresis confirmed this to be the case. Preliminary indications have shown that the sequence of plasmid DNA isolated using the Biometra Magnetic Bead Kits is readable at over 900 bases. The DNA eluted from the DEAE-Magarose is readily collected by precipitation with cold ethanol, giving a visible pellet. In certain cases other ion-exchange based plasmid DNA isolation kits require the use of isopropanol precipitation, which although effective does give a less clearly visible DNA pellet which may result in yield losses due to practical difficulties in handling this pellet.

Table 2
DNA binding properties of DEAE-Magarose

DNA source	Volume of beads used (μl)	DNA yield (μg)	A_{260}/A_{280} ratio
pBluescript	150	8.2 ($n=5$)	1.94
Salmon sperm	100	14.0 ($n=10$)	ND[a]

[a] ND: Not Determined.

The oligo-dT Magarose was shown to bind our fluorescent oligo-dA probe, presumably through hybridisation as previously suggested [14]. In our assay system ca. 40% (w/w) of the oligo-dA present in the starting solution was recovered using the oligo-dT Magarose (Table 3), corresponding to an oligo-dA binding capacity of ca. 75 μmol/g oligo-dT Magarose. Our initial study into the isolation of mRNA from baby hamster cell total RNA, indicated the presence of mRNA by agarose gel electrophoresis. We are unable to quantify the yield of mRNA but at least 1 μg would be needed for visualisation in this manner. On the premise that 1–5% (w/w) of total cellular RNA is mRNA, then from a total of 168 μg RNA a yield would be in the range ca. 2–10 μg. We collected 80 μl of mRNA containing eluate of which 10 μl was electrophoresed, which correlates to a yield in the expected range.

In this study, we present a new paramagnetic agarose matrix, which when derivatized with appropriate ligands adsorbs nucleic acids. Agarose lends itself to derivatization with a wide range of ligands, in order to effect a broader range of bioseparations. We have previously demonstrated the variation between a range of commercially available ion exchangers when screened comparatively [8]. It is reasonable to conclude that these differences were not only due to the functional groups but also to the matrix itself [8]. In the field of nucleic acid purification, the interaction between the nucleic acid and the base matrix is not understood, suffice to say that DNA will bind to an underivatised silica support [16,17], presumably through hydrogen bonds. Agarose is a hydrophilic polysaccharide and once derivatised may possibly exhibit a mixed mode interaction. Given that each nucleic acid is by definition different, then their mode of interaction with an adsorbent surface may consequently be subtly different. Notwithstanding the handling benefits of a magnetic

Table 3
Fluorescein-labelled oligo-dA_{15} binding to oligo-dT Magarose

Stage of experiment	[F-dA_{15}] (μM)
Adsorbate	0.375
Non-bound	0.154
Wash 1	0.053
Wash 2	0.026
Eluate	0.150

separation, Magarose offers the molecular biologist another matrix which may offer selectivity benefits over competitive media when isolating specific nucleic acids, issues which are of importance if purity and quality of the nucleic acids is a pre-requisite.

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