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Application of magnetite and silica–magnetite composites to the isolation of genomic DNA

James I. Taylor, Carolyn D. Hurst, Martin J. Davies*, Niki Sachsinger, Ian J. Bruce

Department of Chemical and Life Sciences, University of Greenwich, Wellington Street, London SE18 6PF, UK

Abstract

Magnetite and silica–magnetite composites were used as adsorbents for the isolation of genomic DNA from maize kernels. Two methods are described for the preparation of silica–magnetite composites, both of which afford higher yields of genomic DNA than when using magnetite alone, or a commerically available kit. DNA isolated using silica–magnetite was suitable for use in further applications such as polymerase chain reaction amplification and enzyme digestion. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Magnetically driven separation techniques using magnetisable solid-phase supports (MSPS) have become commonplace in recent years, proving advantageous to a number of biotechnological applications such as immunoassay, cell sorting and enzyme purification [1]. Also, ventures such as the human genome mapping project, construction of national forensic DNA databases, and the advent of DNA-based diagnostics have driven demand for simple, rapid and efficient methods for the isolation of DNA from a number of cellular sources [2–4]. In several recent reports, we have described the preparation and characterisation of MSPs based on the polysaccharide agarose [5,6], and the application of anion-exchange derivatives of agarose–MSPS to nucleic acid isolation [7,8].

Most recently, we reported the application of the magnetic component of these supports — magnetite — to the isolation of plasmid DNA from bacterial cell lysates [9]. In this method, DNA was adsorbed to the support under high salt conditions, and recovered directly in water for immediate downstream application. These conditions are similar to those used with silica-based purification systems, in which adsorption of double stranded DNA at the silica surface is thermodynamically favoured under high salt or chaotropic conditions. We now report the preparation of silica–magnetite composites, and the application of both these and magnetite itself to the isolation of genomic DNA from a plant source, namely, maize kernels.

*Corresponding author. Fax: +44-20-8331-8405.

E-mail address: m.j.davies@gre.ac.uk (M.J. Davies).

2. Experimental

2.1. Materials

All reagents and solvents were obtained from Fisher Scientific (Loughborough, UK) and were used without further purification. Reagents for use in DNA isolation and analysis were of molecular

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biology grade. RNAse was obtained from Sigma (Poole, UK). Restriction endonucleases and reaction buffers were obtained from Roche (Lewes, UK).

2.2. Preparation of magnetite

Magnetite (Fe_3O_4) was prepared by a modification of the method of Sugimoto [10]. Iron(II) sulfate heptahydrate (354.2 g, 1.27 mol) was dissolved in 3 l of degassed deionised water in a 5 l reaction vessel equipped with overhead stirrer, nitrogen inlet, thermometer and air condenser. The solution was heated to 90°C with stirring, and a solution of potassium nitrate (80.9 g, 0.80 mol) and potassium hydroxide (188.6 g, 3.36 mol) in degassed, deionised water (1 l), which had been preheated to 65°C was added to the solution of iron(II) sulfate. The mixture was stirred for a further hour at a temperature of 92–93°C, with constant purging of the reaction mixture with a slow stream of nitrogen. After this time a black precipitate of magnetite had formed, and the reaction vessel was allowed to cool to room temperature. The suspension was transferred to a large flat-bottomed vessel, and placed on a slab magnet to sediment the magnetite. The supernatant was aspirated using a suction line, then the magnetite resuspended in deionised water (1 l). After stirring for 1 min, the suspension was replaced on the magnet to sediment the magnetite again. This procedure was repeated until the pH of the supernatant was between 7 and 8. Yield of precipitated magnetite was calculated by removing known aliquots of the suspension and drying to constant mass in a vacuum oven at 60°C. This procedure would routinely yield ~40 g of magnetite. The washed magnetite was stored in deionised water at a suspension concentration of 40 mg ml^{-1}. As previously reported, the magnetite was examined by scanning electron microscopy (SEM) and Fourier transform infrared spectrophotometry (FT-IR). It was found to have a mean particle size of 150 nm, and was judged to be at least 99% pure magnetite by FT-IR when compared to an authentic sample of magnetite.

2.3. Preparation of silica–magnetite composites

2.3.1. By deposition of silica from a solution of silicic acid (silicic acid method) [11]

Sodium silicate (8.3 g) was dissolved in deionised water (200 ml) and 170 ml of this solution passed through a column containing Amberlite® 120-(H) ion-exchange resin, which had been washed previously with 200 ml of each of the following: hot deionised water, hydrochloric acid (3 *M*), cold deionised water. The first 20 ml of sodium silicate solution was allowed to pass uncollected to flush the column. The remainder of the eluent was retained and its pH raised to 9.5 using a portion of the stock sodium silicate solution. A 133-ml volume of this solution was added to a stirred mixture of the magnetite suspension (30 ml) and tetramethylammonium hydroxide (TMA) (25%; 19 ml). The pH of this suspension was 12.5. The pH of the suspension was lowered over the period of ~1 h by the dropwise addition of hydrochloric acid (0.5 *M*) until the pH reached 10.0. The suspension was stirred for a further 2 h, then washed with a solution of TMA (pH 10; 2×2 l) followed by deionised water (5×2 l) until the pH of the supernatant was neutral. The silica–magnetite complex was stirred in deionised water at a suspension concentration of 40 mg ml^{-1}.

2.3.2. By hydrolysis of tetraethoxysilane (TEOS method) [12]

Magnetite suspension prepared above (50 ml) was placed in a round-bottomed flask and allowed to settle. The supernatant was removed, and an aqueous solution of tetraethoxysilane (TEOS; 10% (v/v), 230 ml) was added, followed by glycerol (200 ml). The pH of the suspension was lowered to 4.6 using glacial acetic acid, and the mixture then stirred and heated at 90°C for 2 h, under a nitrogen atmosphere. After cooling to room temperature, the suspension was washed sequentially with deionised water (2×500 m l), methanol (2×500 ml) and deionised water (5×500 ml) The silica magnetite composite was stored in deionised water at a concentration of 40 mg ml^{-1}.

2.4. Analysis of silica content of silica –magnetite composites [13]

Silica content was measured by a hydrofluoric acid digestion method. This was carried out by Butterworths Labs. (Teddington, UK).

2.5. Isolation of genomic DNA from maize kernels using magnetite and silica–magnetite [14]

Ground maize kernels (20 mg) were placed in a

1.5-ml microcentrifuge tube, and resuspended by vortexing in 0.1 *M* Tris–HCl buffer (pH 8.0) containing 0.05 *M* EDTA and 0.5 *M* NaCl (200 μl). An 8-μl volume of 10% (w/v) sodium dodecylsulfate and RNAse A (10 mg ml^{-1}; 89 μl) were added, the sample vortexed and incubated at 65°C for 10 min. The tube was placed on ice for 5 min, 5 *M* potassium acetate (13.5 μl) was added and the sample vortexed once more. The tube was placed on ice for a further 20 min, and the resulting suspension centrifuged at 14 000 *g* for 20 min at 4°C. The supernatant was removed and transferred to a fresh 1.5-ml microcentrifuge tube, and an equal volume of 20% (w/v) polyethylene glycol (PEG) 8000 in 4 *M* NaCl was added, followed by 250 μl of the magnetite or silica–magnetite suspension. The mixture was agitated gently at room temperature for 5 min, then the suspension immobilised using a magnetic stand. The supernatant was removed and magnetite or silica–magnetite washed with 50% (v/v) aqueous ethanol for 5 min. The suspension was again immobilised using the magnetic stand, and the supernatant discarded. Adsorbed DNA was eluted from the magnetite or silica–magnetite by addition of sterile water (200 μl) and incubation with gentle agitation at room temperature for 5 min. The particles were immobilised again, and the eluent removed and retained. A 30-μl aliquot of the eluted DNA was analysed by agarose gel electrophoresis on a 0.8% agraose gel in Tris–borate buffer [15]. Positive control DNA was isolated using the commercially available DNEasy Plant Mini Kit (Qiagen, Crawley, UK).

2.6. Restriction endonuclease digestion

A 20-μl volume of the eluted DNA solution was mixed with the manufacturer's reaction buffer (3 μl), sterile water (6 μl) and incubated with the restriction endonuclease Eco R1(1 μl, 10 units) at 37°C for 18 h. The digestion mixture was analysed directly by agarose gel electrophoresis.

2.7. Polymerase chain reaction (PCR) ampification of a 226 base-pair region of the maize invertase gene

This was performed using primers reported by Ehlers et al. [16], using a modified PCR thermal cycling protocol [17].

2.8. Visualisation of DNA on agarose gels, and DNA yield calculation

After electrophoresis, gels were immersed in deionised water containing 0.001% (w/v) ethidium bromide for 20 min, and then viewed using a UV transilluminator and GEL-BASE PRO gel-imaging system, and densitometry software (UVP, Cambridge, UK). Using this software, the intensities of stained DNA bands could be converted to a yield of isolated DNA, by comparison with the intensities of bands due to known amounts of DNA (50, 100, 200, 300, 400 and 500 ng), which were run alongside.

3. Results and discussion

The performance of magnetite, and silica–magnetite composites were compared in the isolation of genomic DNA from maize kernels. Magnetite was prepared by a modification of the method of Sugimoto [10], to allow control of the magnetite particle size. The methods used for the preparation of silica–magnetite composites were based on the previously reported procedures of Butterworth et al. [11], in which the pH of a solution of sodium silicate is lowered in the presence of magnetite, and the method of Whitehead et al., in which TEOS is hydrolysed in the presence of magnetite [12].

The protocol used for plant genomic DNA isolation was based upon published literature methods with the inclusion of an adsorption step in the presence of sodium chloride and PEG 8000. Under these conditions, adsorbed DNA can be eluted directly into water for immediate applications, without the need for precipitation.

We found that when using magnetite as solid-phase adsorbent, yields of recovered genomic DNA from maize kernels were equivalent to those obtained using a commercially available method (DNEasy, Qiagen), up to 1 μg per 20 mg of kernels (as judged by intensity of DNA bands in ethidium bromide stained gels). Agarose gel analysis of supernatants remaining after adsorption showed that no DNA was

present, implying that all available DNA has been adsorbed by the magnetite. However, this yield of recovered DNA is low when compared to that which, although of inferior quality, could be obtained by precipitation of DNA from the crude lysate. We concluded that significant amounts of DNA still remained adsorbed to the magnetite. Prolonged elution at room temperature or elevated temperature (65°C) did not release the DNA, and a partial recovery (~100 ng) could only be achieved by washing the magnetite with 1 *M* sodium chloride. This implies that the adsorption/desorption mechanism varies depending on the conditions under which it takes place, as we have previously observed that plasmid DNA could be recovered in good yield from bacterial cell lysates when magnetite is used as adsorbent [9]. Furthermore, this suggests that adsorption and desorption is dependent upon other factors than simply presence of high salt or chaotropes (to promote adsorption) and elution in water (for desorption). We are currently undertaking detailed studies of the interaction of DNA with magnetite, and will report our findings in due course.

Following this result, we reasoned that it would be desirable to sequester the magnetite in a matrix material that would prevent it from interacting with DNA in solution, but which would still allow the (high salt-adsorption)/(water-desorption) strategy to be followed. The obvious material to select was silica, and thus we prepared silica–magnetite composites by the two methods described above. Silica–magnetite samples were examined by SEM, which indicated that no change in particle size had occurred during the deposition process, and no other surface changes were observed when compared to magnetite.

When either of these materials were used in the DNA isolation protocol, recovered yields of DNA were superior to those obtained with magnetite or the commercial kit, typically 2.0 to 2.5 μg of DNA per 20 mg of maize kernels, as judged by UV spectrophotometry at 260 nm, or preferably, comparison of intensity of DNA bands in ethidium bromide stained agarose gels. The improved recovery of genomic DNA using silica–magnetite as adsorbent confirms that the magnetite surface has been modified with silica, and that the magnetite core has been largely sequestered. However, we still observed that small amounts of DNA could be recovered in a second elution using sodium chloride, indicating that the magnetite surface has not been completely shielded. In an attempt to overcome this, we prepared silica–magnetite composites by either method where four consecutive layers of silica were deposed on the magnetite surface. Their silica content was analysed by hydrofluoric acid digestion (Table 1). Composites prepared by the silicic acid method showed a small increase in silica content after consecutive deposition. Those prepared by the TEOS method had higher silica content, and there was a marked difference between the content for the composite with a single layer, and those with two, three or four layers. These supports were also tested in the DNA isolation protocol and all performed identically (Fig. 1) Again, however, a small but significant amount of adsorbed DNA could be recovered by sodium chloride elution. This suggests that even after four consecutive depositions, magnetite is still displayed at the surface of these composites. In a separate set of experiments, the preparation and use of a silica–silica composite confirmed that this effect was due to the magnetite alone. The silicic acid method was used to depose a layer of silica onto silica gel in suspension, and this material was used to extract high-molecular-mass double-stranded DNA (λ phage DNA) from lysate solutions mixed with 4 *M* NaCl–20% PEG 8000, which had been spiked with known amounts of this DNA, followed by elution into water. This material displayed none of the additional desorption characteristics of magnetite, or silica–magnetite (results not shown).

The A_{260}/A_{280} ratios of all samples of isolated

Table 1
Silica content of silica–magnetite composites; values are average of four replicate analyses

	No. of deposed silica layers	Silica content (%, m/m)
Composite prepared by silicic acid method	1	1.40
	2	1.60
	3	1.73
	4	1.92
Composite prepared by TEOS method	1	1.76
	2	22.42
	3	33.17
	4	41.60

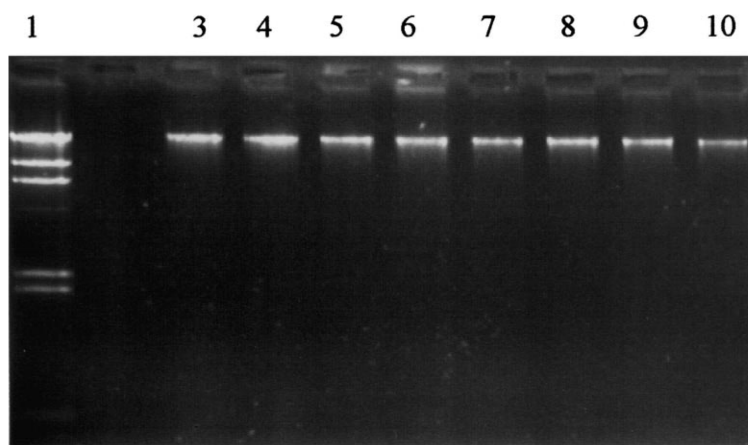

Fig. 1. Agarose gel electrophoresis of genomic DNA isolated from maize kernels. Lanes: 1=DNA molecular mass marker (λ phage DNA/*Hin*d III digest; 3–6=DNA isolated using silica–magnetite prepared by silicic acid method, having one, two, three or four consecutively deposed layers of silica; 7–10=DNA isolated using silica–magnetite prepared by TEOS method, having one, two, three or four consecutively deposed layers of silica.

plant genomic DNA were calculated as a first measure of purity. The results indicated that the quality of DNA extracted using silica–magnetite prepared by the silicic acid method was marginally higher than that isolated using TEOS silica–magnetite. All silica–magnetite samples yielded DNA of an equivalent, or higher purity to that isolated using the DNEasy kit (Table 2).

The A_{260}/A_{280} ratios for the majority of samples are relatively low, indicating contamination with other species, the possible identity of which is discussed later. However, despite this and the observed secondary adsorption effects, the yield of DNA recovered using either silica–magnetite composite was more than sufficient for further application. Furthermore, isolated DNA functioned satisfactorily in PCR amplification (Fig. 2) and there was no obvious difference in the performance of DNA isolated using composites with up to four consecutively deposed layers of silica. In all extractions, the genomic DNA band migrated an equivalent distance to the 23 kilobase-pair (kb) band of the λ phage DNA/*Hin*d III digest molecular mass marker.

However, in another application — digestion of recovered DNA with a restriction endonuclease — a

Table 2
Yields and A260/A280 ratios of plant genomic DNA isolated from maize kernels using silica–magnetite composites, magnetite, and commercially available DNEasy kit; values are average of triplicate isolations

	No. of deposed silica layers	DNA yield/μg (A_{260}/A_{280})
Composite prepared by silicic acid method	1	2.5 (1.40)
	2	2.3 (1.68)
	3	2.4 (1.60)
	4	2.0 (1.50)
Composite prepared by TEOS method	1	2.3 (1.40)
	2	2.3 (1.52)
	3	2.3 (1.37)
	4	2.4 (1.34)
Magnetite		1.0 (1.30)
DNEasy		1.0 (1.33)

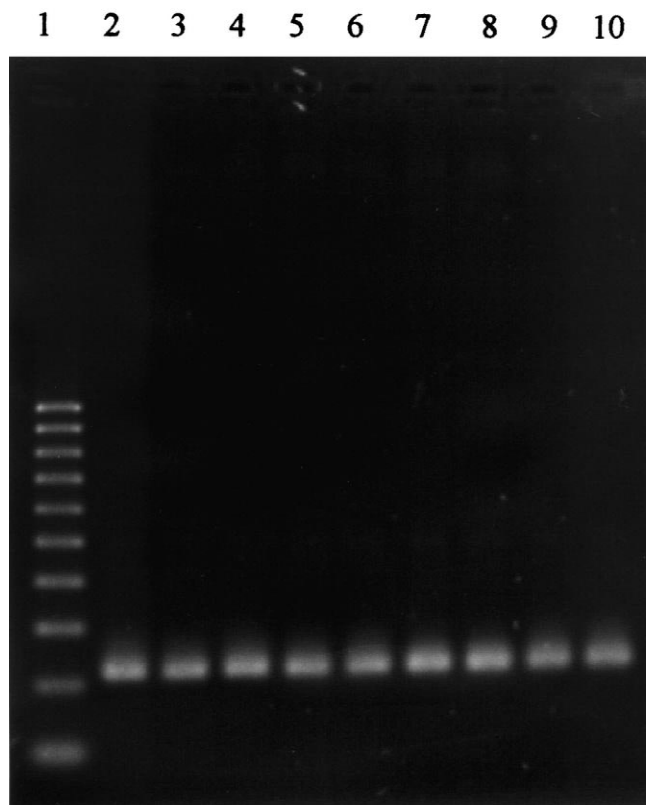

Fig. 2. Agarose gel electrophoresis of 226-base pair (bp) fragment of maize invertase gene obtained by PCR amplification of DNA isolated using silica magnetite composites. Lanes: 1 = DNA molecular mass markers; 2–5 = PCR product from DNA isolated using silica–magnetite prepared by silicic acid method, having one, two, three or four consecutively deposed layers of silica; 6–9 = PCR product from DNA isolated using silica–magnetite prepared by TEOS method, having one, two, three or four consecutively deposed layers of silica; 10 = PCR product from DNA isolated using DNEasy kit (Qiagen).

difference was observed in performance. Digestion of the DNA recovered using each consecutively deposed sample was performed, and analysed by agarose gel electrophoresis (Fig. 3). DNA isolated using any of the composites prepared by the silicic acid method was completely digested after an overnight incubation, whilst DNA isolated using the composite with one or two layers of silica deposed using the TEOS method was not fully digested. DNA from TEOS composites having three or four layers was completely digested. In the isolation method used here, the materials that may effect efficiency of digestion, which may be carried over with eluted DNA, are plant polysaccharide, terpenoids, phenols and tannins [13,18]. It is known that whilst the adsorption of double stranded DNA to silica is thermodynamically favourable under high salt conditions, adsorption of other species such as protein, single stranded nucleic acids and polysaccharides is disfavoured [19,20]. As has already been discussed, all composites prepared may still display magnetite at the surface even after consecutive deposition, and the results of the digestion experiment point to interaction of magnetite with polysaccharide or other plant isolates as the source of this contamination. Since the TEOS composites having three or four silica layers yield DNA that is completely digested, the incomplete digestion of DNA isolated using the one- or two-layer TEOS composite cannot be due to polysaccharide carried over by to an interaction with

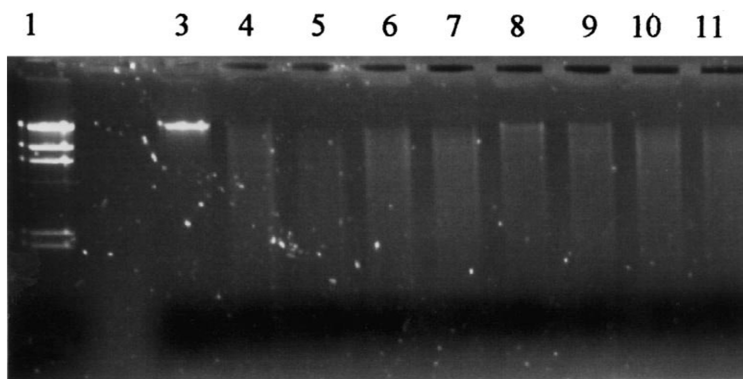

Fig. 3. Agarose gel electrophoresis of restriction endonuclease digestion of DNA isolated from maize kernels. Lanes: 1=DNA molecular mass markers (λ phage DNA/*Hin*d III digest; 3=undigested reference DNA; 4–7=digestion of DNA isolated using silica–magnetite prepared by silicic acid method, having one, two, three or four consecutively deposed layers of silica; 8–11=DNA isolated using silica–magnetite prepared by TEOS acid method, having one, two, three or four consecutively deposed layers of silica.

silica. Once the magnetite has been sufficiently covered, the amount of polysaccharide that may be carried over with eluted DNA is not great enough to hinder the digestion reaction.

This result also indicates differences between the two deposition methods — the silicic acid method appears to be more efficient in covering a higher proportion of the magnetite surface than the TEOS method, as the sample with one deposed silica layer yields DNA which can be fully digested. Although the TEOS method produces composites with higher silica content, it does not appear to be as efficient at surface coverage. This in turn may be a consequence of the different types of silica that each produces [13]. This will be an important consideration when applying composites prepared by different methods to isolation of DNA from other sources, using different conditions. Other workers have reported the influence that varying the type of silica or silicaceous material can have on yield, purity and applications of isolated DNA [18,21].

In conclusion, we prepared silica–magnetite composites which were used to isolate plant genomic DNA. These materials display dual adsorption properties that demand further investigation, which we are currently pursuing. Notwithstanding these effects, the yield and quality of DNA obtained is sufficient to allow further downstream application. We also demonstrated in other experiments that these composites can adsorb high-molecular-mass DNA from spiked lysate solutions, indicating that they can be applied to the extraction of genomic DNA from other cellular sources. For example, these composites were recently used for the isolation of genomic DNA from whole blood [22].

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