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Harnessing metal ion affinity for the purification of plasmid DNA

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Abstract

The recent surge in plasmid DNA (pDNA) vaccine research has generated demand for an efficient large-scale pDNA purification process. In this study, three process intensification strategies harnessing the interactions of pDNA, RNA and endotoxin with immobilised and/or free transition metal ions were investigated for the purification of plasmid pcDNA3.1D, containing dengue fever antigenic gene (NS3), from the alkaline cell lysate. In the first process scheme, alkaline cell lysate was applied to Cu^{2+}–iminodiacetic acid (IDA) for simultaneous removal of RNA and endotoxin. Subsequent addition of $CuCl_2$ to the supernatant afforded selective precipitation of pDNA, resulting in substantially purified pDNA at an overall recovery yield of 92% with significant reduction in RNA (96%) and endotoxin (> 99%). In the second process scheme, pDNA, RNA and endotoxin were first isolated from other impurities in alkaline cell lysate by $CuCl_2$ precipitation. Addition of EDTA to the precipitated pellets selectively solubilised pDNA and RNA while endotoxin remained insoluble. Subsequent application of solubilised pDNA and RNA to Cu^{2+}–IDA resulted in highly purified pDNA with almost complete removal of RNA and any residual endotoxin (∼ 100% pDNA recovery, ∼ 100% removal of RNA and endotoxin). In the third process scheme, RNA and endotoxin were first removed from alkaline cell lysate by $ZnCl_2$ precipitation. pDNA in the supernatant was then recovered by $CuCl_2$ precipitation (64% pDNA recovery, 86% RNA and > 99% endotoxin removal). In essence, the combination of metal ion ($CuCl_2$) precipitation, followed by immobilised metal affinity chromatography (IMAC) employing Cu^{2+}–IDA, provides a great potential to achieve significant intensification of pDNA purification process with improved efficiency and throughput.

Keywords: Plasmid DNA purification; Immobilised metal affinity chromatography (IMAC); Metal ion precipitation; Process intensification

1. Introduction

Plasmid DNA (pDNA) vaccine involves the cloning of an antigen into the plasmid (an extrachromosomal, double-stranded DNA molecule, Schmidt et al., 2001) which will prime both humoral and cellular immune responses against the antigenic organism following vaccination (Gurunathan et al., 2000). Potential advantages of pDNA vaccine over conventional vaccine include induction of long-lived immune responses (Robinson et al., 1997) and the ease of storage/transportation (Gurunathan et al., 2000). Hence, immense interest exists in the development of large-scale pDNA purification process to supply pDNA for clinical trials and market (Horn et al., 1995). In addition, preferred pDNA purification processes should not utilise animal-derived enzymes (e.g. ribonuclease A, lysozyme and proteinase K) or toxic chemicals (e.g. cesium chloride and ethidium bromide) that are either not recommended or not approved in production of pDNA for therapeutic applications (Eon-Duval and Burke, 2004).

Current large-scale pDNA purification processes generally comprise of alkaline cell lysis, cell debris separation, affinity precipitation, adsorption and buffer exchange before clinical-grade pDNA is obtained (Prather et al., 2003). For affinity precipitation, the use of compacting agents, such as spermine (Mourich et al., 2003; Murphy et al., 1999), spermidine

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(Murphy et al., 1999; Stepanov and Nyborg, 2003), high concentration salt (e.g. 2.5–5 M LiCl (Mourich et al., 2003; Stepanov and Nyborg, 2003), 1.4 M calcium chloride (Eon-Duval et al., 2003) and 2.5 M ammonium sulphate (Diogo et al., 2000)) and polycation (Wahlund et al., 2004), to selectively isolate pDNA or impurities (e.g. RNA and endotoxin) from the alkaline cell lysate was reported. However, problems associated with current affinity precipitation methods include: (1) the requirement of high salt condition for impurity precipitation (Diogo et al., 2000; Eon-Duval et al., 2003; Mourich et al., 2003; Stepanov and Nyborg, 2003), hence requiring additional desaltation; (2) incomplete impurity precipitation (Diogo et al., 2000; Eon-Duval et al., 2003) commonly followed by the need to incorporate additional purification steps such as tangential flow filtration (TFF) for the removal of low molecular weight RNA (Eon-Duval et al., 2003).

Apart from selective precipitation of pDNA or impurities, purification of pDNA using size exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC), affinity chromatography and anion exchange chromatography (AEC) have often been employed (Diogo et al., 2005). For most chromatographic columns, disadvantages include poor selectivity of the column for pDNA or impurities and/or low capacity of the chromatographic beads for large pDNA molecule (Diogo et al., 2005). Recently, new chromatographic bead designs were explored, aiming to increase the selectivity and binding capacity of the beads for pDNA or impurities. The use of biporous hydrophobic adsorbent beads (100% recovery of pDNA, 100% removal of RNA and proteins, endotoxin removal not determined) (Li et al., 2005), anion exchange lid beads with non-charged exterior coupled with positively charged pore surface (69% recovery of pDNA, 100% removal of RNA, incomplete removal of proteins, endotoxin removal not determined) (Kepka et al., 2004) and monoliths anion exchange beads (incomplete removal of proteins) (Urthaler et al., 2005) were reported. With the biporous and monoliths anion exchange beads, the chromatography was conducted following the use of $\sim$ 2.5 M ammonium sulphate to effect partial removal of RNA and proteins from the alkaline cell lysate (Li et al., 2005; Urthaler et al., 2005). With the lid beads, ultra/diafiltration and two-phase system thermoseparation (50% ethylene oxide–50% propylene oxide vs. dextran T500) were required before chromatographic separations (Kepka et al., 2004).

Owing to the inefficiency of the various unit operations, numerous process steps (e.g. membrane filtration, several affinity precipitation steps and adsorption chromatography) are required to isolate pDNA, hence rendering the purification process to be time and cost prohibitive. This is partly due to the similar nature of pDNA and impurities (e.g. RNA and endotoxin), mostly negatively charged, and thus causing the separation of pDNA from impurities to be a challenge even when the capacity of chromatographic column for pDNA or impurities is increased.

In our previous study (Tan et al., 2007), immobilised metal affinity chromatography (IMAC), in particular Cu^{2+}–iminodiacetic acid (IDA) and Ni^{2+}–IDA, was reported to be highly efficient in the direct removal of RNA and endotoxin from the alkaline cell lysate. In addition, free transitional metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} and Fe^{3+}) exhibited varying extent of interactions with pDNA and RNA, hence possibly applicable for selective precipitation of nucleic acids from the alkaline cell lysate. For instance, Zn^{2+} showed the greatest selectivity between pDNA and RNA with pDNA remaining in the solution when almost full precipitation of RNA was achieved, thereby showing possibility of using Zn^{2+} for selective precipitation of RNA from the alkaline cell lysate. In the present study, the focus is to develop an efficient pDNA purification process by harnessing the specific interactions of pDNA, RNA and endotoxin with immobilised and/or free metal ions.

2. Materials and methods

All experiments were conducted at room temperature unless otherwise stated.

2.1. Materials

IDA-Chelating Sepharose Fast Flow (Amersham, 17-0575-01) was the IMAC resin employed. Reagents used were tris (Numi, Singapore), sodium acetate trihydrate (Riedel-de Haen, 32318), sodium chloride (HCS, S6001-1-1000), EDTA disodium dihydrate (Duchefa Biochemie, E0511.0500), Seakem LE agarose (Cambrex, 50004), ethidium bromide solution (Bio-rad, 1610433), 1 kb DNA ladder (Promega, G5711) and Blue/Orange Loading Dye 6x (Promega, G1881). Baker's yeast RNA (R6750), cupric chloride dihydrate (C6641), zinc chloride (Z0152) and imidazole (I2399) were from Sigma. Slide-A-Lyser Mini Dialysis Units with a nominal MWCO of 10 kDa (69 576) and SnakeSkin Pleated Dialysis Tubing with a nominal MWCO of 3.5 kDa (68 035) were supplied by Perbio Science; QIAprep Spin Miniprep Kit (27104) (miniprep) by Qiagen.

2.2. Fermentation and alkaline cell lysis

DH5-α mutant harbouring plasmid pcDNA3.1D, a 7.3 kb high copy plasmid (100–200/cell) which contains 1.8 kb dengue fever antigenic gene (NS3), was kindly provided by Bioprocessing Technology Institute (Singapore). Cells were grown on 12 g/l yeast extract (BD BBL 211929), 6 g/l tryptone (BD Bacto), 5 g/l glucose (Sigma, G8270), 6 g/l K_2HPO_4 (US Biological, P5100) and 0.48 g/l $MgSO_4$ (Sigma, M7506) using a 30 l fermenter (B. Braun Biotech International). Cell lysate was obtained using the fermentation conditions and alkaline cell lysis procedures as described previously (Tan et al., 2007).

2.3. Batch adsorption study for RNA and endotoxin

IDA-Chelating Sepharose Fast Flow was washed with water (2 × 5 settled resin volumes (SV)), charged with 100 mM $CuCl_2$ or $NiCl_2$ (2 SV), washed with water (5 × 5 SV), and finally, equilibrated with equilibrating buffer (3 × 5 SV). Baker's yeast RNA was dissolved in Tris buffer (20 mM tris, pH 7),