Journal of Chromatography A, 1217 (2010) 2262–2266

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Clearance of host cell impurities from plasmid-containing lysates by boronate adsorption

A. Gabriela Gomes, Ana M. Azevedo, M. Raquel Aires-Barros, D. Miguel F. Prazeres*

IBB – Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 28 October 2009
Received in revised form 3 February 2010
Accepted 8 February 2010
Available online 16 February 2010

Keywords:
Gene therapy
Plasmid DNA
RNA
Lipopolysaccharides
Genomic DNA
Affinity process
Phenyl boronate
Adsorption
Purification
Chromatography

ABSTRACT

The ability of boronate adsorption to clear *Escherichia coli* impurities directly from plasmid-containing lysates (~pH 5.2) was evaluated. Results show that 3-aminophenyl boronate (PB) controlled pore glass (CPG) is able to adsorb not only those species that bear *cis*-diol groups (RNA, lipopolysaccharides-LPS), and are thus able to form covalent bonds with boronate, but also *cis*-diol-free proteins and genomic DNA (gDNA) fragments, while leaving most plasmid DNA in solution. Control runs performed with phenyl Sepharose and with PB-free CPG beads ruled out hydrophobic interactions with the phenyl ring and non-specific interactions with the glass matrix, respectively, as being responsible for RNA and gDNA adsorption. In batch mode, up to 97.6 ± 3.1% of RNA, 94.6 ± 0.8% of proteins and 96.7 ± 11.7% of gDNA were cleared after 30 min, with a plasmid yield of 64%. In fixed-bed mode, most of the plasmid was recovered in the flowthrough (96.2 ± 4.0%), even though the RNA (65.5 ± 2.8%), protein (84.4 ± 1.3%) and gDNA clearance (44.7 ± 14.1%) were not as effective. In both cases, the LPS content was removed to a residual value of less than 0.005 EU/ml. The method is fast and straightforward, circumvents the need for pre-treatment of the feed and may contribute to shorten plasmid purification processes, as the treated streams can proceed directly to the final polishing steps.

1. Introduction

Gene therapy is an alternative for the prevention and treatment of genetic defects and acquired diseases [1]. Plasmid DNA (pDNA) vectors can be used in this context to deliver therapeutic transgenes into the target cells [2]. One of the keys for the development of such pDNA biopharmaceuticals is the availability of scalable production methods. After amplification in *Escherichia coli* (*E. coli*), pDNA must be separated from proteins (~55%, w/w), RNA (~20%, w/w), lipopolysaccharides (LPS, ~3%, w/w) and genomic DNA (gDNA, ~3%, w/w). The major challenges after alkaline lysis, the key disruption step in pDNA manufacturing, are the removal of RNA, LPS, and traces of gDNA, and the isolation of supercoiled pDNA isoforms from other less active variants [3,4]. Although several processes are available, new purification methods are actively pursued to facilitate the manufacturing of kilogram-amounts of pDNA with the stringent quality requirements stipulated by the FDA [5,6].

Affinity techniques use ligands that bind specifically and reversibly to the target molecules by intermolecular forces (e.g. ionic bonds, hydrogen bonds, van der Waals forces). Phenyl boronate (PB) ligands are able to form a pair of covalent bonds with molecules containing *cis*-diols via a reversible esterification reaction [7,8] (Fig. 1). In acidic solutions, boronic acids adopt a trigonal planar form which can revert to a tetrahedral boronate anion upon hydroxylation in alkaline conditions [9]. Both the acid and its conjugate base can bind to a diol compound. However, since the equilibrium constant for the tetrahedral form (K_{tet}) is usually higher than that of the trigonal form (K_{trig}), complexes are less stable in acidic conditions [7]. Most ligands used in boronate affinity adsorption are aromatic and thus also able to establish hydrophobic and aromatic π–π interactions. Secondary ionic interactions between boronates and diols are also possible through coulombic attraction or repulsion effects, hydrogen bonding via the hydroxyl groups and coordination interactions [9]. Boronate adsorption has been used to separate carbohydrates, nucleic acids and glycoproteins [9,10]. This work explores the ability of PB resins to adsorb RNA (via the 1,2-*cis*-diol in ribose) and LPS (via the *cis*-diols in the saccharide moiety) from plasmid-containing lysates. DNA is not expected to bind since deoxyribose lacks the 2′-hydroxyl group [9].

2. Materials and methods

2.1. Chemicals

Controlled porous glass (CPG) beads (74–125 μm, pore size ~1000 Å) with immobilized 3-aminophenyl boronic acid (ProSep®-

* Corresponding author. Tel.: +351 218419133; fax: +351 218419062.
E-mail address: miguelprazeres@ist.utl.pt (D.M.F. Prazeres).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.02.015

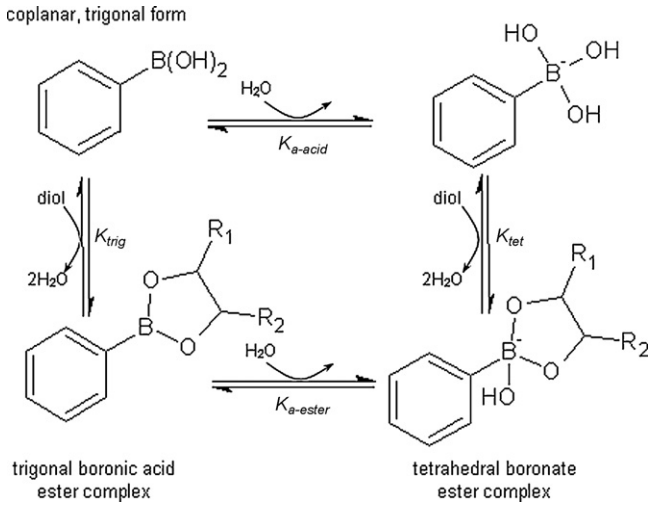

Fig. 1. Equilibria for boronic acid complexation with diols in aqueous solutions. Both the boronic acid and the boronate anion reversibly bind diols, forming a trigonal boronic acid ester (equilibrium constant K_{trig}) and a tetrahedral boronate ester (equilibrium constant K_{tet}), respectively.

PB) and with no derivatization were from Millipore (Bedford, MA, USA). All chemicals were of analytical grade and solutions were prepared in MilliQ water.

2.2. Plasmid DNA production

E. coli DH5α cells harbouring the 6050 bp pVAX1-LacZ plasmid (Invitrogen, Carlsbad, CA) were cultivated for 8 h in flasks with 250 ml of Luria Bertani medium and 30 μg/ml of kanamycin at 37 °C and 250 rpm. Cells were harvested with an OD600 nm ≈3.4 and alkaline lysis was performed as described earlier [11].

2.3. Phenyl boronate adsorption

PB adsorption was performed at room temperature in fixed-bed and batch modes using 3-aminophenyl boronate CPG beads. In batch experiments 560 μl of settled PB resin (equilibrated with water) and lysates (750 μl) were incubated in 1.5 ml microcentrifuge tubes for 1, 5 and 30 min. End-over-end mixing was performed during adsorption and elution. At each time point, the correspondent tube was centrifuged and the supernatant (~750 μl) was withdrawn. The material adsorbed was eluted in two consecutive steps with 750 μl of 1.5 M Tris (pH 8.7). After 1 h, beads were centrifuged, supernatants withdrawn and nucleic acids measured at 260 nm. Three independent experiments were performed and each time point was assayed in triplicate. For fixed-bed experiments, a column (5 mm i.d.) was packed with 746 μl of PB resin and connected to an Äkta purifier (GE Healthcare, Uppsala, Sweden). After equilibration with water (1.0 ml/min), 1 ml of lysate was injected, the column was washed with 10 ml of water and then eluted with 1.5 M Tris, pH 8.7. Fractions (0.5 ml) were pooled in two final samples (flowthrough and elution peaks), eluate absorbance was monitored at 260 nm. All runs were performed in triplicate, as three independent assays.

2.4. Analytics

Samples were analyzed by hydrophobic interaction HPLC [11,12]. A 15 PHE PE HIC column (4.6 mm × 10 cm) from GE Healthcare was equilibrated with 1.5 M ammonium sulfate in 10 mM Tris, pH 8.0 (1 ml/min). Lysate and samples from batch experiments were diluted 5-fold in this same buffer, whereas other samples were analyzed with no dilution. Following injection (30 μl), isocratic elution was performed with the equilibration buffer for 1.4 min and then with 10 mM Tris, pH 8.0 buffer for 0.9 min. At 2.3 min, the column was re-equilibrated with 1.5 M ammonium sulfate. Plasmid concentration was determined from a calibration curve (2.5–100 μg/ml). A HPLC purity was obtained by dividing the pDNA peak area by the total peak area in the chromatogram [11,12]. The amount of RNA removed was also estimated on the basis of the method.

Total protein in samples was quantified in triplicate using the BCA Protein kit from Pierce (Rockford, EUA). LPS concentration was assessed using the kinetic-QCL Limulus amoebocyte lysate (LAL) assay kit from Biowhittaker (Walkersville, USA). Genomic DNA in the feed lysate and in samples collected after batch and fixed-bed adsorption was estimated by real time PCR as described previously [13]. Samples were analyzed by agarose electrophoresis according to [12].

3. Results

Plasmid-containing alkaline lysates were contacted with PB matrix in batch and fixed-bed mode. The volumetric ratio of lysate to PB matrix was kept constant (~1.3) to facilitate comparison.

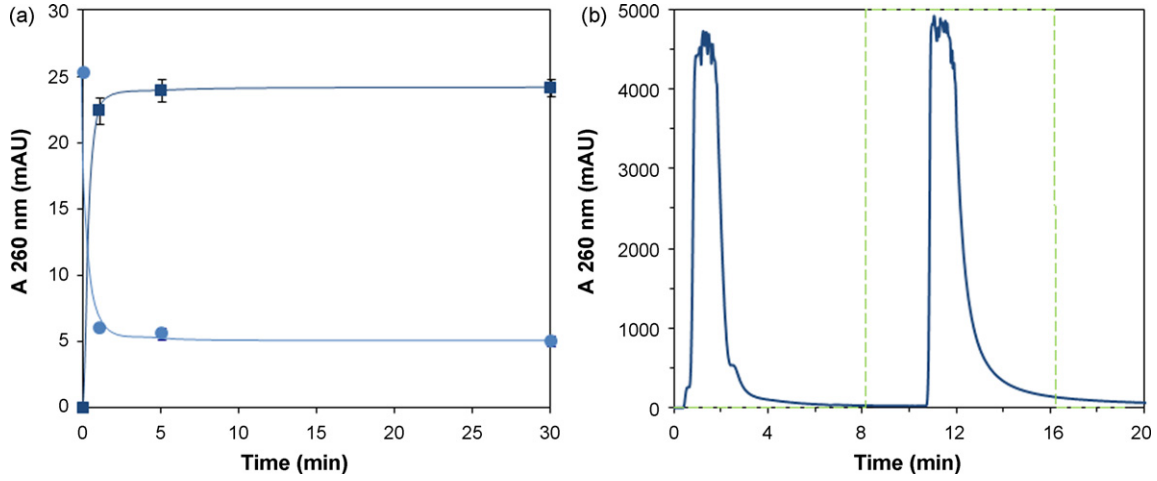

Fig. 2. Clearance of nucleic acids from plasmid-containing *E. coli* lysates by PB adsorption in batch (a) and fixed-bed mode (b). (a) The time course evolution of nucleic acids (measured by absorbance at 260 nm) in the liquid phase is shown over time of adsorption (●) and elution (■) for a 750 μl lysate sample. (b) The chromatogram shows the absorbance at 260 nm of the eluate stream after injection of 1 ml of lysate (solid line). The dashed line indicates the 1.5 M Tris step gradient.