Separation and Purification Technology 66 (2009) 397–404

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Partition of plasmid DNA in polymer–salt aqueous two-phase systems

Frank Luechau*, Tau Chuan Ling[1], Andrew Lyddiatt[2]

Biochemical Recovery Group, Department of Chemical Engineering, School of Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:
Received 29 July 2008
Received in revised form
28 November 2008
Accepted 4 December 2008

Keywords:
Partition behaviour
Plasmid DNA (pDNA)
Aqueous two-phase systems (ATPS)
High molecular weight (HMW)
Low molecular weight (LMW)

ABSTRACT

The partition of plasmid DNA (pDNA) in polyethylene glycol (PEG)–phosphate aqueous two-phase systems (ATPS) is presented. A high molecular weight (HMW) and a low molecular weight (LMW) polymer, PEG-1450 and -300, were used in combination with di-potassium hydrogen phosphate. The experimental results demonstrated that the plasmid pTX0161 displays a varied partition behaviour in PEG–phosphate ATPS. In HMW PEG (PEG-1450–phosphate systems), pDNA partitioned to the bottom phase only. In LMW PEG (PEG-300–phosphate systems), pDNA partitioned to all of the phases with respect to the phase composition, system temperature and concentration of lysate used in the ATPS. In systems with volume ratios higher than one, pDNA was mainly recovered in the top phase. For volume ratios between 0.5 and 1, pDNA mainly partitioned to the interface. In systems with volume ratios below 0.5, most of the pDNA was recovered in the bottom phase. For temperatures between 4 and 25 °C, the partition to the top phase decreased whereas partition to the interface steadily increased. At 25 °C, over 80% of pDNA was recovered in the interface. The partition to the bottom phase increased steadily with increasing temperatures up to 40 °C and the partition to the interface decreased. At 20 °C, the recovery of pDNA in the interface gradually increased and reached a maximum at 60% (w/w) lysate with 80% recovery recorded. At 25 °C, over 80% of pDNA was recovered in the interface from lysate concentrations greater than 35% (w/w). At 30 °C, the top phase preference changed to an interface preference between 0 and 20% (w/w) lysate.

1. Introduction

In gene therapy, a gene is introduced to specific cells in a patient to produce therapeutic biomolecules, which are normally proteins. As a result, gene therapy can be applied to the treatment or prevention of genetic and acquired diseases [1,2]. The number of protocols has increased recently and they can be categorised [3] based on the method of administration (*ex vivo* and *in vivo*) and the gene expression system (viral and non-viral). Plasmid DNA (pDNA) based gene therapy belongs to the category of non-viral systems [4]. Generally, their production is considered more scalable, the process is easier to control and monitor and quality control testing is deemed easier than that for viral vectors [5]. Due to the development of molecular therapies (e.g., DNA vaccination and therapeutical uses) have increased the need of larger amounts of pDNA [6]. Process routes for the large-scale manufacture of pDNA have been developed [7], but there is always room for improvement and a need [8,9] for alternative process routes.

The potential advantages of extraction methods based on aqueous two-phase systems (ATPS) are that they can combine two or more processing steps including cell debris removal, concentration, separation and purification [10–12]. ATPS composed of two polymers have been used to partition nucleic acids [13]. Recently, the partition of pDNA in PEG–salt ATPS has received more attention, probably initiated by an increased demand for pDNA as non-viral gene therapy vectors [14–16]. The apparent advantages of polymer–salt systems over polymer–polymer systems are, firstly, their lower phase viscosities which makes them easier to handle on a larger scale. Secondly, the cost of phase-forming chemicals is much lower for polymer–salt systems when compared with polymer–polymer systems. Up until now, the main drawback of using polymer–salt ATPS for the partition of nucleic acids has been that they seem to be less selective than polymer–polymer systems.

In this paper, the partition of pDNA in di-potassium hydrogen phosphate ATPS is presented. The experiments were carried out with RNA-depleted *Escherichia coli* (*E. coli*) lysate which rendered the interpretation of pDNA partition results easier. For the experiments, a HMW and a LMW polymer, PEG-1450 and -300, were used in combination with di-potassium hydrogen phosphate. The two polymers were chosen as they were expected to have either

* Corresponding author. Current address: B.Braun Melsungen AG, Aesculap Division, Vascular Systems, Sieversufer 8, D-12359 Berlin, Germany.
Tel.: +49 3068989778.
E-mail addresses: frank.luechau@bbraun.com, f_luechau@hotmail.com (F. Luechau).
[1] Current address: Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2] Current address: Newcastle University Business School, Citywall, Citygate, St James Boulevard, Newcastle upon Tyne, NE1 4JH, United Kingdom.

1383-5866/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.seppur.2008.12.003

a weak (PEG-300) or a strong (PEG-1450) steric exclusion effect on pDNA. The influence of the system composition, temperature, lysate concentration and initial pDNA concentration on pDNA in LMW polymer (PEG-300) partition was carried out here.

2. Materials and methods

2.1. Materials

The lysate containing the pDNA vector designated pTX0161 with 7796 base pairs and a molecular weight of 5.06×10^6 Da was kindly provided by Cobra Therapeutics (Keele, UK) and prepared as described in Varley et al. [17]. *E. coli* cell paste containing pTX0161 was produced and kindly provided by Saethawat Chamsart, University of Birmingham, UK. PicoGreen™ double-stranded (ds) DNA quantification reagent was from Molecular Probes, Inc. (Eugene, OR). Ribonuclease A (Type I-AS, from bovine pancreas) and all of the chemicals were of analytical grade and supplied by Sigma–Aldrich, Ltd. (St. Louis, MO, USA). BCA protein assay reagents and bovine serum albumin (BSA) were from Perbio Science Ltd. (Cheshire, UK).

2.2. Alkaline cell lysis

Crude *E. coli* cell lysis was prepared as described in Varley et al. [17]. In order to prepare a total of four volumes of lysate, the following steps were taken. *E. coli* cells were resuspended in one volume of resuspension buffer (50 mM Tris–HCl, 10 mM EDTA, pH 8.0). Two volumes of lysis buffer (155 mM NaOH, 1% (w/v) SDS) were added and mixed for 10 min at ambient temperature. One volume of neutralisation buffer (3 M potassium acetate, 10 mM EDTA, pH 5.5) was then added to the suspension, mixed gently and incubated for up to 1 h at ambient temperature. Precipitated cell debris and flocculated salts were filtered out once through Whatman No. 1 filter paper (Whatman Ltd., Maidstone, UK).

2.3. Preparation of ATPS

All of the ATPS were made up and described on a % (w/w) basis. An ATPS composed of, for example, 15% (w/w) polyethlyene glycol (PEG) 300 and 22% (w/w) di-potassium hydrogen phosphate was designated 15/22. Blank systems contained only phase-forming chemicals, added salts and distilled water. Crude systems contained feedstock such as lysate or *E. coli* cell paste. ATPS were composed of lysate, PEG and potassium hydrogen orthophosphate (di-basic). Lysate which had been prepared with ribonuclease A as described by Varley et al. [17] was virtually RNA-free. In preparation for the partition experiments, the lysate was filtered through a 0.45 μm cellulose acetate sterile filter to remove any insoluble materials. The phase-forming chemicals were added as solids or stock solutions (liquid PEG-300, 50% (w/w) PEG-1450 solution and 50% (w/w) phosphate solution). The pH of ATPS containing cell lysates was adjusted to pH 8 with concentrated HCl.

All of the experiments were carried out under defined temperatures (4–40 °C) in a water bath. ATPS were assembled in a small plastic beaker and mixed on a magnetic stirrer until equilibrium. After initial sampling, the mixture was transferred to a 15 ml centrifuge tube and phase separation was accelerated by centrifugation at $1000 \times g$ for 3 min using a Bench Jouan Centrifuge C422 (Jouan, France). The volumes of top and bottom phases were recorded for the calculation of volume ratios and mass balances. Material at the phase interface was harvested together (inevitably) with small volumes of top and bottom phases and resuspended in a known volume of 10 mM Tris–HCl, 1 mM EDTA, pH 8.0 (TE) buffer. Top and bottom phase samples as well as interface samples were analysed for nucleic acid concentrations.

2.4. Preparation of concentrated pDNA solution

A concentrated pDNA solution was required for a number of studies. Plasmid-rich phases were collected throughout scouting experiments with RNA-poor lysate and pooled together. The pool was dialysed in 12,000 Da molecular weight cut-off (MWCO) tubing (Sigma–Aldrich, Dorset, UK) against TE buffer for 24 h at 6 °C. The pDNA was concentrated by precipitation with PEG-8000. A 30% (w/v) PEG-8000 solution in 1.6 M NaCl was added to a plasmid solution (dialysed against TE buffer) to achieve a final PEG concentration of 10% (w/v) [18,19]. Samples were allowed to stand for 24 h at 6 °C. Nucleic acids were centrifuged down at $10{,}000 \times g$ for 30 min at 4 °C. The supernatant was removed and the precipitated pellet was resuspended with TE buffer.

2.5. pDNA determination

The plasmid DNA concentration was measured with an intercalating dye called PicoGreen™ [20]. Samples were diluted with Tris buffer (50 mM Tris–HCl, 1 mM EDTA buffer pH 7.5) to achieve concentrations between 0.05 and 0.4 μg ml^{-1} pDNA. Samples were mixed with PicoGreen™ stock solution with a ratio of 1:1 (the stock solution was obtained by diluting concentrated PicoGreen™ 500-fold with Tris buffer). The mixture was poured into a clear-sided cuvette and stored for 1 h in the dark at 20 °C. Samples were measured in a PerkinElmer Ltd. luminescence spectrometer LS50B (Beaconsfield, UK) with an excitation at 485 nm and an emission at 520 nm against Tris buffer as a blank. The resulting concentrations were calculated from a calibration curve for pure pTX0161 in Tris buffer. All of the measured results were expressed in (μg pDNA equivalent) ml^{-1}.

3. Results and discussion

3.1. Partition of pDNA in HMW PEG systems

In systems with HMW PEG-1450, virtually all of the pDNA partitioned to the bottom phase. Fig. 1 shows the normalised concentration of pDNA in bottom phases of ATPS having increasing volume ratios. Initially, a clean ATPS composed of 12% (w/w) PEG 1450 and 12% (w/w) di-potassium hydrogen phosphate (designated as 12/12) was chosen as a reference system and its corresponding

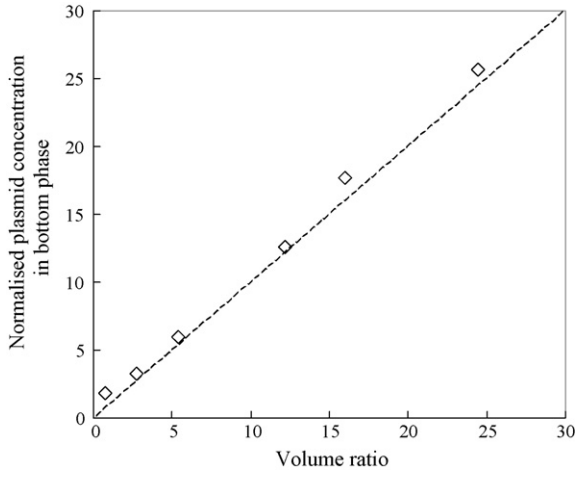

Fig. 1. Concentration of pDNA in PEG-1450 phosphate bottom phases. The concentration of pDNA in bottom phases of PEG-1450 phosphate ATPS with increasing volume ratios was investigated. The pDNA was concentrated in the bottom phase as a result of their one-sided partition behaviour. The measured pDNA concentration in the bottom phase (◊) was normalised, i.e. its concentration was divided by the initial pDNA concentration of the total dispersed ATPS.