Journal of Chromatography B, 878 (2010) 1939–1945

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Affinity recovery of lentivirus by diaminopelargonic acid mediated desthiobiotin labelling

Rongjun Chen[a], Najeem Folarin[b], Vincent H.B. Ho[c], David McNally[c], David Darling[b], Farzin Farzaneh[b], Nigel K.H. Slater[c,*]

[a] Centre for Molecular Nanoscience, School of Chemistry, University of Leeds, Leeds LS2 9JT, UK
[b] Department of Haematological and Molecular Medicine, Guy's, King's and St Thomas' School of Medicine, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NU, UK
[c] Department of Chemical Engineering and Biotechnology, University of Cambridge, New Museums Site, Pembroke Street, Cambridge CB2 3RA, UK

ARTICLE INFO

Article history:
Received 3 March 2010
Accepted 17 May 2010
Available online 24 May 2010

Keywords:
7-DAPA
Desthiobiotin
Metabolic biotinylation
Streptavidin
Monomeric avidin
Vesicular stomatitis virus glycoprotein pseudotyped lentivirus
Affinity chromatography
Gene therapy

ABSTRACT

Desthiobiotin-tagged lentiviral vectors have been metabolically produced by DBL producer cells in a 7,8-diaminopelargonic acid (7-DAPA) dependent manner for envelope independent, single-step affinity purification. 7-DAPA, which has little or no affinity for avidin/streptavidin, was synthesised and verified by NMR spectroscopy and mass spectrometry. By expressing the biotin acceptor, biotin ligase and desthiobiotin synthase bioD, DBL cells converted exogenous 7-DAPA into membrane-bound desthiobiotin. Desthiobiotin on the DBL cell surface was visualised by confocal microscopy and the desthiobiotin density was quantified by HABA-avidin assay. Desthiobiotin was then spontaneously incorporated onto the surface of lentiviral vectors produced by the DBL cells. It has been demonstrated by flow cytometry that the desthiobiotinylated lentiviruses were captured from the crude 7-DAPA-containing viral supernatant by Streptavidin Magnespheres® and eluted by biotin solution efficiently whilst retaining infectivity. The practical, high yielding virus purification using Pierce monomeric avidin coated columns indicates a highly efficient biotin-dependent recovery of infectious lentiviruses at 68%. The recovered lentiviral vectors had a high purity and the majority were eluted within 45 min. This 7-DAPA mediated desthiobiotinylation technology can be applied in scalable production of viral vectors for clinical gene therapy.

1. Introduction

Lentiviral vectors are attractive for gene therapy applications and their clinical promise for the treatment of HIV/AIDS has been demonstrated [1]. Amongst other problems, the progress in lentiviral gene therapy has been hampered by the requirement for production of purified lentiviral vectors with high titre. Lentiviral vectors are often purified using conventional multi-step downstream processing methods including density-gradient centrifugation [2], precipitation [3], filtration [4], size exclusion and ion-exchange chromatography [5–8]. These low specificity processes are time-consuming and some are scale limited and generally result in low recoveries of infectious viral particles. Antibody and heparin affinity chromatography have enabled a specific separation of herpes simplex viruses [9], adeno-associated viruses [10] and lentiviruses [11] from impurities generated by host cells. However, both of these techniques have potential constraints in manufacturing. Antibody production, purification and immobilisation are costly at large scale and for each virus type a different antibody would be required [9]. In addition, many cellular proteins are known to associate with heparin, hence the use of a heparin column requires the incorporation of a specific step to remove contaminating heparin-binding proteins [10].

We have a long-standing interest in the use of affinity chromatography as a single-step capture method for the generic recovery of viral vectors by exploiting streptavidin–biotin interactions. Nesbeth et al. [12] engineered a novel human 293T based packaging cell line BL15, which metabolically produces spontaneously biotin-tagged lentiviral vectors requiring only biotin in the culture medium. This metabolic biotinylation technology facilitates highly efficient affinity-mediated paramagnetic-particle and chromatographic capture of viral particles but a medium change prior to viral harvest is obligatory [12–14]. However, its value in manufacturing is limited because: (i) the crude supernatants containing biotinylated viruses are contaminated with competing free-biotin that reduce the efficiency of viral capture; (ii) the high-affinity binding of biotin to streptavidin (dissociation constant $K_d \sim 10^{-15}$ M) [15,16] makes it unsuitable for processes requiring efficient elution of viruses from affinity supports under physiological conditions [13]. To address these two limitations, this work

* Corresponding author. Tel.: +44 1223 762953; fax: +44 1223 334796.
E-mail address: nkhs2@cam.ac.uk (N.K.H. Slater).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.05.019

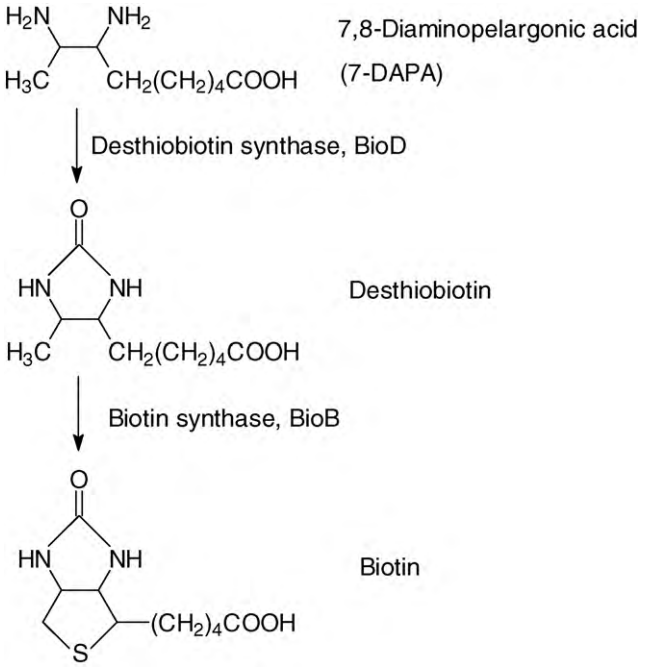

Fig. 1. The biosynthetic pathway of biotin.

demonstrates that 7,8-diaminopelargonic acid (7-DAPA) mediated desthiobiotin labelling of the lentiviral surface enables reversible binding to avidin/streptavidin.

In *Escherichia coli*, the precursor 7-DAPA is synthesised into desthiobiotin by the enzymatic action of synthase bioD (Fig. 1) [17]. A new packaging cell line, DBL, has been developed by cloning and expressing synthase bioD in BL15 cells (data published elsewhere). In summary the bioD was cloned, sequenced and ligated into the Eukaryotic expression vector pIREShyg3. The use of a suboptimal cloning site 3′ to the Internal Ribosome Entry Site (IRES) allows hygromycine B dependent selection of transfected cells expressing high levels of insert derived protein, in this case BioD. This allows desthiobiotinylation of the DBL cell surface followed by the production of desthiobiotin-tagged lentiviral vectors when 7-DAPA is present in the culture medium. This development enables two major processing enhancements. Firstly, 7-DAPA does not bind avidin/streptavidin [18], so desthiobiotin-tagged lentiviruses can be captured directly from crude supernatants without the need for pre-treatments to remove free avidin-binding impurities. Secondly, desthiobiotin has a lower affinity for avidin/streptavidin (K_d ~10^{-13} M) than biotin [16,19], and the desthiobioitin-labelled lentiviral vectors are therefore amenable to gentle elution strategies. Monomeric avidin, which reportedly has a lower affinity binding to biotin (K_d ~10^{-7} M) than streptavidin [20], is demonstrated as a replacement to streptavidin in this paper to further improve the virus purification process.

2. Materials and methods

2.1. Materials

Streptavidin Magnespheres® paramagnetic particles (1 mg mL^{-1}) were supplied by Promega (Southampton, UK). Barium hydroxide monohydrate was obtained from Fisher (Loughborough, UK). RPMI-1640 medium (without L-glutamine), Dulbecco's modified Eagle medium (DMEM), CellMask™ plasma membrane stain, NuPAGE® Novex® Bis–Tris 4–12% Mini Gels, Novex® MES SDS Running Buffer (20×) and Novex® LDS sample buffer (4×) were purchased from Invitrogen (Paisley, UK). Pierce SilverSnap® Stain Kit and Pierce monomeric avidin coated columns were purchased from Thermo Scientific (Rockford, USA). Desthiobiotin, foetal bovine serum (FBS), dialysed FBS (10,000 Da molecular weight cut-off), Dulbecco's phosphate buffered saline (D-PBS), penicillin–streptomycin solution (10,000 U mL^{-1} penicillin and 10 mg mL^{-1} streptomycin), L-glutamine solution (200 mM), puromycin, blasticidin, hygromycin, avidin-FITC, 4-hydroxyazobenzene-2-carboyxlic acid (HABA)–avidin reagent, trypsin–EDTA solution (0.5 g L^{-1} porcine trypsin and 0.2 g L^{-1} EDTA·4Na) and Hanks' balanced salt solution (HBSS) were obtained from Sigma–Aldrich (Dorset, UK).

2.2. Synthesis of 7-DAPA

7-DAPA was chemically synthesised according to the procedure illustrated in Fig. 2. A 20-mL Biotage Microwave Vial (Biotage, Sweden) was added with 160 mg of desthiobiotin, 16 mL of deionised water, 3.53 g of barium hydroxide monohydrate as a catalyst and a magnetic stir bar, and then sealed. The reaction proceeded at 158 °C in an Initiator Microwave Synthesiser (Biotage, Sweden) for 7.5 h. The sample was then centrifuged twice to remove insoluble impurities. The supernatant was saturated with carbon dioxide and the precipitate was removed by centrifugation. The resulting supernatant was acidified with 1 M H_2SO_4 (pH ~6.5) to remove residual barium ions, and then lyophilised to fine white powder (yield = 62%). The product was stored at 4 °C.

2.3. Structural characterisation

^{13}C NMR spectra of 7-DAPA (in D_2O) and desthiobiotin (in d_6-DMSO) were obtained on a Bruker Advance 500 MHz spectrometer (Bruker Biospin GmbH, Germany) at room temperature. High-resolution mass spectrometry (HRMS) was carried out using a Micromass Quadrupole-Time of Flight spectrometer. ^{13}C NMR (500 MHz, D_2O) for 7-DAPA: δ 183.9, 53.7, 49.0, 37.3, 29.9, 28.1, 25.3, 24.4, 13.6 ppm; HRMS (m/z) for $[C_9H_{21}N_2O_2]^+$, calculated: 189.1603, found: 189.1610.

2.4. Cell culture

Human 293T based BL15 packaging cells [12] were grown in DMEM supplemented with 10% (v/v) biotin-free FBS, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 2.5 μg mL^{-1} blasticidin and

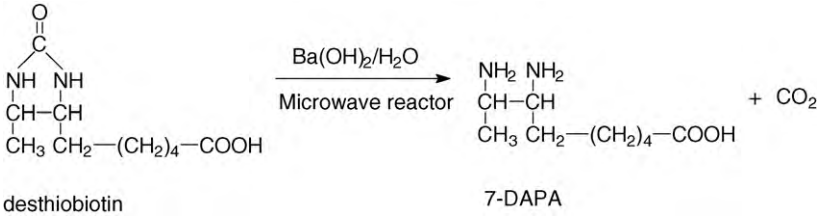

Fig. 2. Synthetic scheme of 7,8-diaminopelargonic acid (7-DAPA).

2.5 μg mL^{-1} puromycin. In this cell line the extracellular domain of a fragment of the low-affinity nerve growth factor receptor (LNGFR) [21] has been fused to a biotin acceptor peptide (BAP) [22]. The expression of LNGFR-BAP with the bacterial birA gene [23] in these cells has enabled the endogenous metabolic biotinylation of a specific lysine residue in the BAP region. The biotin is thus transported to the cell surface as a membrane anchored affinity moiety and incorporated onto the membrane of the lentiviral particles produced by these cells. The DBL packaging cell line has been developed by cloning and expressing synthase bioD alongside birA and LNGFR-BAP in BL15 cells (data published elsewhere). In summary the bioD was cloned, sequenced and ligated into the Eukaryotic expression vector pIREShyg3. The use of a suboptimal cloning site 3′ to the Internal Ribosome Entry Site (IRES) allows hygromycine B dependent selection of transfected cells expressing high levels of BioD. The bacterial birA gene already expressed in the cells would then covalently tag the BAP region with desthiobiotin which was converted from 7-DAPA by the enzymatic action of BioD, thus allowing production of desthiobiotin-tagged lentiviral vectors. DBL cells were grown in DMEM supplemented with 10% (v/v) biotin-free FBS, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 2.5 μg mL^{-1} blasticidin, 2.5 μg mL^{-1} puromycin and 200 U mL^{-1} hygromycin. K562 human immortalised myelogenous leukaemia cells were maintained in RPMI-1640 medium and TE671 human rhabdomyosarcoma cells were maintained in DMEM. Both of these sets of medium were supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. The four types of cells were split three times per week. Cells were maintained in a humidified incubator at 37 °C and 5% CO_2.

2.5. Laser scanning confocal microscopy

To visualise the cell surface desthiobiotinylation, 4×10^5 DBL cells were cultured on a 35-mm glass-bottom culture dish (MatTek, USA) with 2 mM 7-DAPA for 96 h. The spent medium was removed gently and the cells were incubated in 1 mL of fresh complete DMEM containing 5 μg mL^{-1} CellMask™ plasma membrane stain at 37 °C for 5 min. The stain was removed and the cells were washed gently three times with D-PBS. 2 mL of complete DMEM containing 80 μg mL^{-1} avidin-FITC was then added to the cells at 4 °C for 30 min. The FITC was aspirated and the cells were washed with D-PBS twice. An SP5 laser scanning confocal microscope (Leica, Germany) was used to image the desthiobiotinylated DBL cells. The avidin-FITC and CellMask™ plasma membrane stain were excited using laser lines of 488 and 543 nm respectively.

2.6. HABA-avidin assay

The extent of 7-DAPA mediated desthiobiotinylation on the DBL cells was determined by using the HABA-avidin assay [24,25]. The DBL cells were cultured with 7-DAPA at the described concentration for the indicated time periods. The spent medium was then removed and the cells were rinsed with D-PBS. The absorbance of fresh HABA-avidin solution, which contains 0.3 mM HABA, 0.45 mg mL^{-1} avidin, 0.01 M HEPES (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid], a buffer with pK_a = 7.5), 0.3 M NaCl, 0.01 M $MgCl_2$ and 0.02% sodium azide, was first measured at 500 nm and 2 mL of the solution was added to the adherent DBL cells in a culture flask and incubated for 5 min. The HABA-avidin solution was then removed and its absorbance was measured at 500 nm. The change in absorbance of the HABA-avidin solution and the relevant cell counts were used to calculate the average number of surface desthiobiotin per cell. For the experimental control, DBL cells (cultured in 7-DAPA free medium) were incubated with HABA-avidin solution.

2.7. Desthiobiotinylation of lentiviral vectors

Vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped lentiviral vectors, which were produced from the 7-DAPA-treated DBL packaging cells, acquired desthiobiotinylated envelope proteins as they budded from the DBL cell membrane. Briefly, the DBL cells were trypsinised and plated at 1×10^6 cells per 90 mm Petri-dish in 10 mL of complete DMEM containing 7-DAPA at the described concentration. After 72 h the cells cultured in 7-DAPA-containing medium were transiently transfected with a combination of green fluorescence protein (GFP) lentivirus (vector, 10.0 μg per dish), delta 8.91 (helper, 7.0 μg per dish) and VSV-G (envelope, 3.5 μg per dish) plasmids by calcium phosphate transfection. After 48 h the lentiviral supernatant was harvested and filtered with 0.45 μm filter for processing and infection of target cells.

2.8. Determination of viral titre

4×10^5 suspension K562 cells were seeded into each well of a 24-well plate containing complete RPMI-1640 medium (1 mL/well containing 4.4 μg mL^{-1} polybrene). The plate was placed into a 37 °C incubator for 1–2 h. Preparations of freely suspended or Streptavidin Magnespheres® bound viruses were titred as described previously [12,26]. Briefly, samples were diluted serially 1:10 in complete RPMI-1640 medium and 100 μL was added to triplicate wells with mixing. After 48 h the suspension K562 cells were split by replacing 900 mL of cell suspension with the same volume of fresh complete RPMI-1640 medium. The plate was returned to culture for a further 96 h after which the colony forming unit (cfu) number was determined by analysis of the percentage of GFP-expressing cells on a BD FACScan flow cytometer (BD Bioscience, USA). The concentration of cfu mL^{-1} was calculated as the number of colonies per well multiplied by the dilution factor. In some case, adherent TE671 cells were chosen as target cells with a seeding density at 5×10^4 cells/well.

2.9. Batch capture and elution of lentviral vectors using Streptavidin Magnespheres®

Streptavidin Magnespheres® were supplied sterile and prepared by washing three times in HBSS. 100 μL of these streptavidin coated paramagnetic particles were added to 3 mL of the harvested 7-DAPA-containing lentiviral supernatant and mixed at room temperature for 2 h using a rotary mixer. The paramagnetic particles were then separated by a Dynal Magnetic Separator (Invitrogen, UK) and the titres (cfu mL^{-1}) of the viral supernatants before and after the contact period and the adsorbed viruses on the Streptavidin Magnespheres® were determined. For the elution study, the desthiobiotinylated lentiviral vectors were desorbed from the Streptavidin Magnespheres® using 0.6, 1 and 3 mM D-biotin (dissolved in pH 7.4 D-PBS) respectively. After the lentivirus-adsorbed paramagnetic particles were washed with HBSS twice, 1 mL of D-biotin solution was added to the adsorbent sample and mixed for 2 h at room temperature using a rotary mixer. The titres of the eluted viruses and the remaining viruses on the Streptavidin Magnespheres® were determined.

2.10. Column-based purification of lentiviral vectors

Pierce monomeric avidin coated columns (2 mL) were selected to test the affinity column-based recovery of desthiobiotin-tagged lentiviral vectors. The columns were equilibrated with PBS buffer, loaded with crude 7-DAPA-containing lentiviral feedstocks by gravity flow, washed with PBS buffer and eluted with 2 mM biotin. Column breakthrough, wash and elution fractions were collected

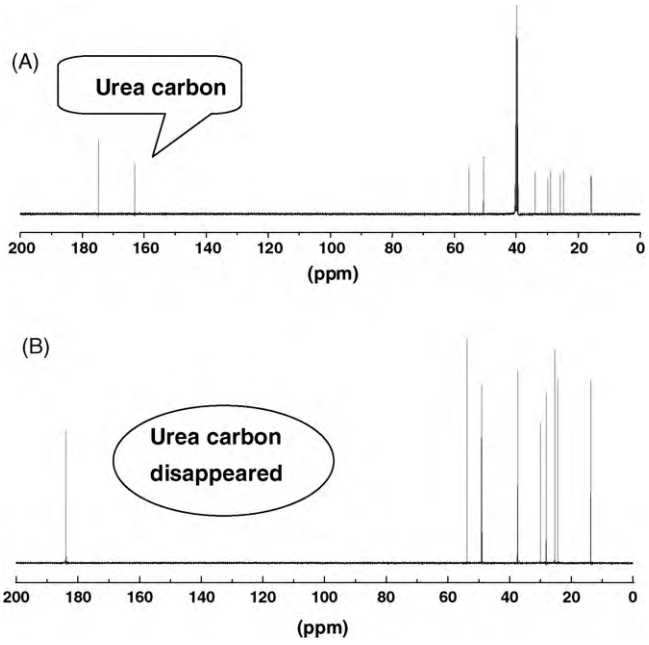

Fig. 3. ^{13}C NMR spectra of (A) desthiobiotin in d_6-DMSO and (B) 7-DAPA in D_2O.

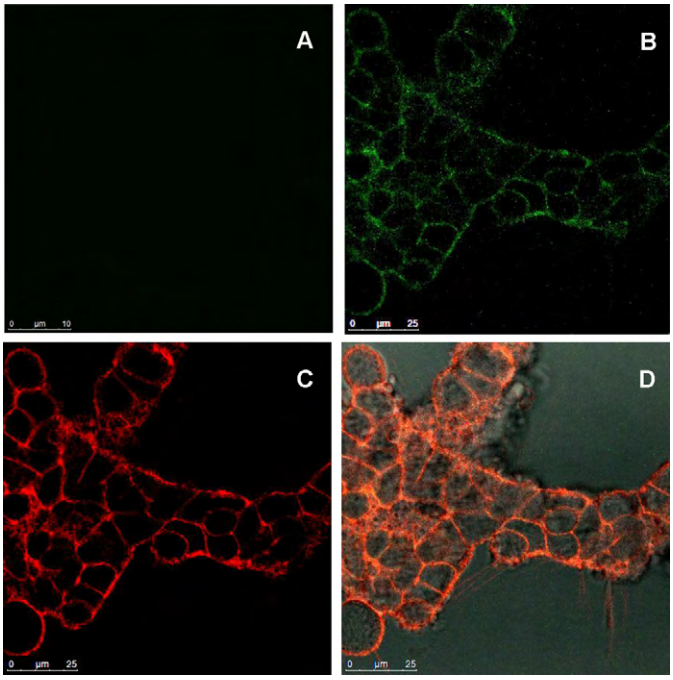

Fig. 4. Laser scanning confocal microscopy images of DBL cells and the control BL15 cells incubated with 2 mM 7-DAPA for 96 h. (A) No detectable fluorescence on BL15 cells after avidin-FITC staining. (B) Desthiobiotin detected on the surface of DBL cells after avidin-FITC staining. (C) DBL cell membranes visualised by CellMask™ staining. (D) Overly of (B) and (C).

and analysed by flow cytometric analysis of the percentage of GFP-expressing cells after 6 days of infection of target cells.

2.11. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

Samples (5 μL) were heated at 90 °C for 30 min using a Stuart block heater (Bibby Scientific, UK) to inactivate viruses. These samples were analysed by SDS-PAGE using NuPAGE® Novex® Bis–Tris 4–12% Mini Gels and XCell Mini-Cell according to the manufacturer's instructions (Invitrogen, UK). The gel was stained with Pierce SilverSnap® Stain Kit according to the manufacturer's instructions (Thermo Scientific, USA) and then photographed on a MEDALight light panel (Morco, UK) using a Cannon EOS 400D Digital SLR Camera (Japan).

3. Results and discussion

3.1. Synthesis and characterisation of 7-DAPA

7-DAPA is not available commercially and so the synthesis of Hoffmann et al. [27] was modified to make 7-DAPA from desthiobiotin in a microwave synthesiser. The purified product was analysed by ^{13}C NMR. Compared to the ^{13}C NMR spectrum of the starting desthiobiotin, it is noticeable that the urea carbon group (δ 162.9 ppm) on the cyclic urea derivative had disappeared in the putative 7-DAPA product (Fig. 3). The molecular weight of 7-DAPA was verified by high-resolution mass spectrometry.

3.2. Desthiobiotinylation of cell surface

The hypothesis that DBL cells with a combination of LNGFR/BAP (acceptor), birA (biotin ligase) and bioD (desthiobiotin synthase) would convert exogenous 7-DAPA into membrane-bound desthiobiotin was tested by confocal microscopy analysis (Fig. 4). Avidin-FITC was able to detect the presence of desthiobiotin (Fig. 4B) and CellMask™ stain was able to locate the position of the cell plasma membrane (Fig. 4C). The overlay of Fig. 4B and C (Fig. 4D) shows that desthiobiotinylation on the surface of DBL cells was successful. No detectable avidin-FITC fluorescence was observed on the parent BL15 cells (Fig. 4A), indicating that DBL cells utilised 7-DAPA as a result of BioD expression.

The density of desthiobiotin on the DBL cell surface was quantified by HABA-avidin assay (Fig. 5). Desthiobiotin can displace HABA in the HABA-avidin complex easily due to its higher affinity binding to avidin ($K_d \sim 3.5 \times 10^{-13}$ M) [16] than HABA ($K_d \sim 6 \times 10^{-6}$ M) [28]. The resulting reduction in absorbance of the HABA-avidin solution at 500 nm can be related to the desthiobiotin concentration. Fig. 5A shows that the average number of desthiobiotin molecules on the cell surface was dependent on the amount of 7-DAPA added to the culture. The extent of desthiobiotinylation was slightly increased at a 7-DAPA concentration lower than 0.5 mM compared to the control DBL cells without the treatment of 7-DAPA. The degree of desthiobiotin labelling on the DBL cell membranes was increased significantly to 1.2×10^9 desthiobiotin molecules per cell relative to the control with increasing 7-DAPA concentration up to 2 mM. Fig. 5B shows that a substantial degree of desthiobiotinylation could be obtained after 24 h. This level increased to a peak at 48 h where further incubation resulted in decreased activity. A control sample in the absence of 7-DAPA was necessary to ascertain the level of background due to non-specific interactions between membrane proteins and HABA [25].

3.3. Virus capture and elution

DBL cells were cultured continuously in 7-DAPA-containing medium and viral labelling continued from transfection up to the time of viral harvest. Fig. 6A shows that the efficiency of viral capture, indicated by the depletion of VSV-G pseudotyped lentivirus from the viral supernatant compared with starting viral titre, increased gradually up to 52% with increasing 7-DAPA concentration to 2 mM. It is interesting to note that the variation of the virus capture efficiency is consistent with that of the extent of desthiobiotinylation (Fig. 5A). In addition, Fig. 6B shows that the lower extent of desthiobiotinylation at 0.5 mM of 7-DAPA resulted in the higher

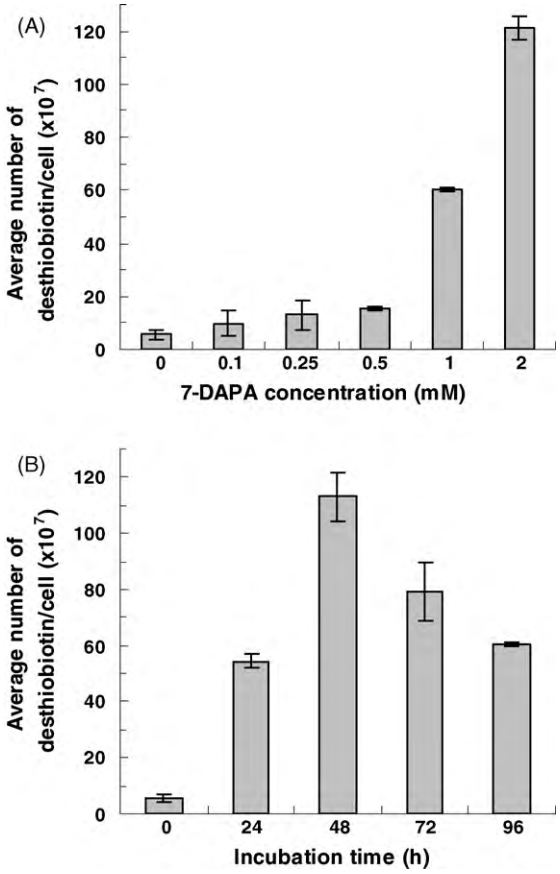

Fig. 5. Characterisation of the extent of desthiobiotinylation on the surface of DBL cells by using the HABA-avidin assay. (A) 7-DAPA concentration-dependent desthiobiotinylation after 96 h. (B) Incubation duration-dependent desthiobiotinylation of DBL cells contacted with 1 mM DAPA. The error bars represent standard deviations of triplicate samples.

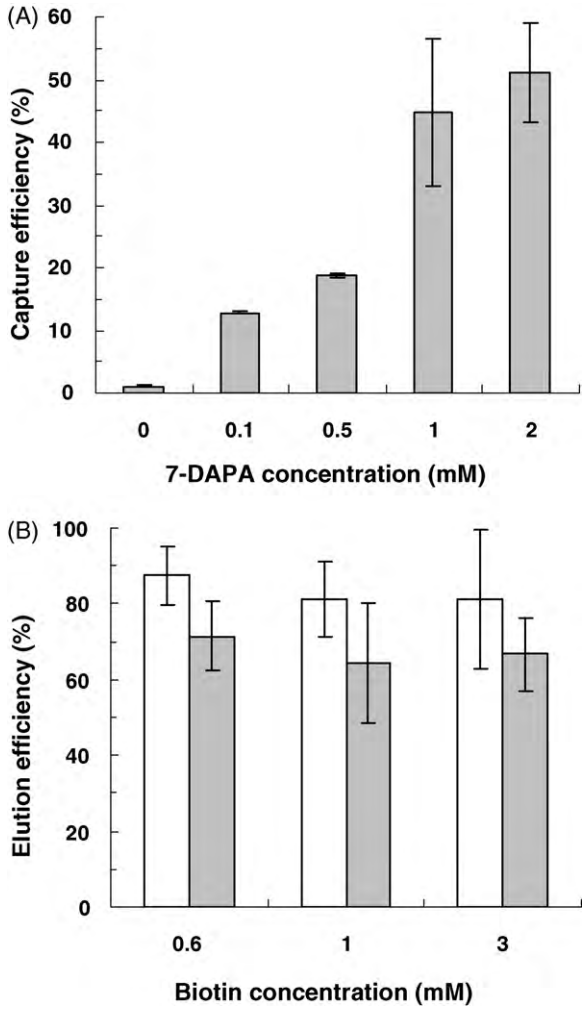

Fig. 6. (A) The influence of the concentration of 7-DAPA contacted with DBL cells for 96 h upon the capture of desthiobiotinylated VSV-G pseudotyped lentiviral vectors on Streptavidin MagneSpheres®. (B) Elution efficiencies of the lentiviral vectors produced by DBL cells incubated with 7-DAPA at 0.5 (blank columns) and 2 mM (grey columns) by using different concentrations of biotin. 7-DAPA-containing lentiviral supernatant (3 mL) from cultures of DBL cells was contacted with 100 μL of Streptavidin MagneSpheres® for 2 h at room temperature using a rotary mixer. Samples (100 μL) were taken from the viral supernatants before and after the contact period and assayed to infect TE671 cells (5×10^4 cells mL^{-1}). Viral titre (cfu mL^{-1}) was determined by flow cytometric analysis of the percentage of GFP-expressing cells after 6 days of infection. The capture efficiency is expressed as the percentage of decrease in titre of the viral supernatant. In addition, The Streptavidin MagneSpheres® were washed with HBSS, resuspended in 10 μL of RPMI 1640, and then assayed to infect K562 cells (4×105 cells mL^{-1}). The titre of adsorbed viruses (cfu mL^{-1}) on the Streptavidin MagneSpheres® before elution was determined. For the elution test, the Streptavidin MagneSpheres® were resuspended in 1 mL of 0.6, 1 and 3 mM biotin (dissolved in pH 7.4 D-PBS) for 2 h at room temperature using a rotary mixer. The titre of remaining viruses on the Streptavidin Magnespheres® was determined similarly by infection of K562 cells, and then the elution efficiency was calculated. The error bars represent standard deviations of triplicate samples.

elution of the desthiobiotin-tagged lentiviral vectors. It has been suggested by Williams et al. [13] that the ligand density on the viral surface is an important parameter for viral capture and elution, and can be adjusted by varying the degree of labelling of the packaging cells. Fig. 6B shows that over 65% of these adsorbed desthiobiotinylated lentiviral vectors can be eluted by using biotin solution, which is significantly higher compared to biotinylated viral vectors (~5%) [13].

The capture and elution profiles of the desthiobiotin-labelled VSV-G pseudotyped lentiviral vectors were thus used as a basis for the design of a column strategy. 12 mL of DBL-derived crude lentiviral supernatant (2.13×10^6 viral vectors), which was harvested without the pre-treatment to remove 7-DAPA, was applied to a 2-mL Pierce monomeric avidin coated column. As shown in Fig. 7A, 89% of lentiviral vectors (1.89×10^6 viral vectors) were captured by the column. The elution efficiency using 22 mL of 2 mM biotin, as determined by the biotin-dependent recovery of captured lentiviruses without initial batch contact, reached 77% (1.45×10^6 viral vectors). Analysis of the total lentiviruses eluted compared to the total load indicates an overall yield of purified lentiviral vectors of 68%. For comparison, recovery of active viral vectors post-purification of approximately 30% is taken to be common for currently proposed purification schemes [29]. Fig. 7B shows that 67% of the captured lentiviruses (1.26×10^6 viral vectors) can be eluted within 45 min, indicating that 7-DAPA dependent purification of crude feedstocks, where no avidin-binding impurities are required to be removed prior to purification, can achieve very fast and efficient viral recovery. Another recent report of purification of biotinylated-adenovirus by monomeric avidin coated SoftLink™ resin necessitated 4 h initial batch contact followed by overnight elution in order to improve capture and elution efficiencies [30]. The recovered lentiviral vectors were analysed by using the SDS-PAGE analysis. Lanes 5–8 in Fig. 8 have no detectable protein impurities, suggesting that the recovered viruses contain much less protein contaminants than the virus-containing cell culture supernatant. The protein impurities present in the crude virus-containing cell culture supernatant (Lane 2) were found in the supernatant after adsorption (Lane 3) and washing fraction (Lane 4). This indicates an efficient protein clearance and

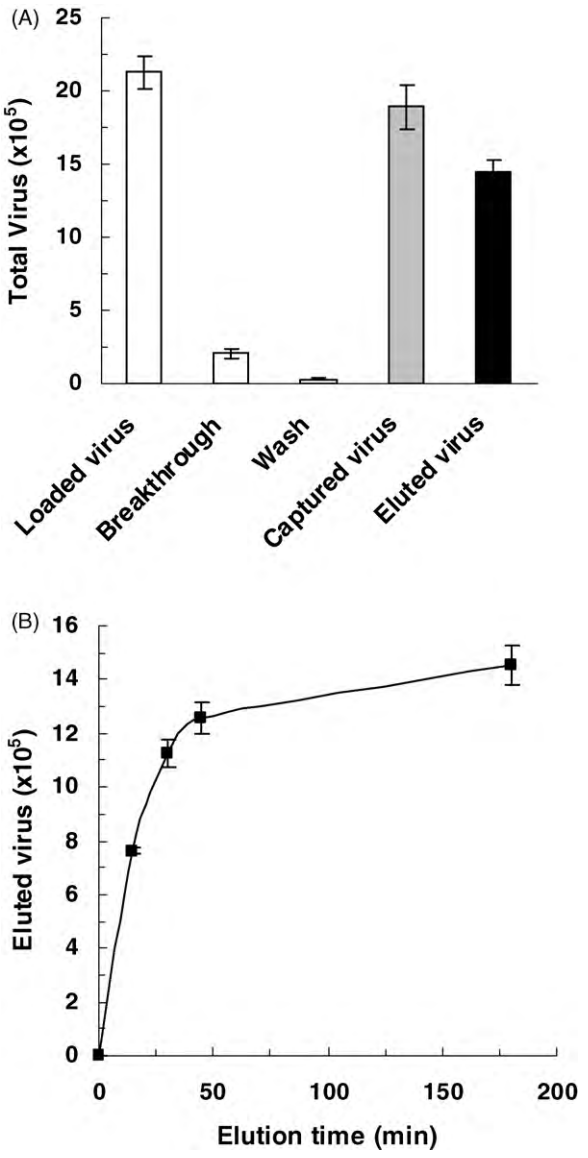

Fig. 7. Column-based purification of desthiobiotinylated lentiviral vectors produced by DBL cells contacted with 2 mM 7-DAPA for 96 h. (A) Pierce monomeric avidin coated columns (2 mL) were loaded with 12 mL of crude 7-DAPA-containing lentiviral feedstocks by gravity flow, washed with 8 mL of PBS buffer, and eluted with 22 mL of 2 mM biotin. Column fractions of breakthrough, wash and elution were analysed by infection of K562 cells (4×10^5 cells mL^{-1}) and the titre of each fraction was determined by flow cytometric analysis of the percentage of GFP-expressing cells after 6 days of infection. (B) Elution profile of these lentiviral vectors in the monomeric avidin coated column at a flow rate of 0.4 mL min^{-1}. The error bars represent standard deviations of triplicate samples.

it is noteworthy that individual viral proteins are not visible on this gel.

The lentiviral vectors are produced by the human 293T based packaging cell line DBL, enabling the lentiviral surfaces to be decorated with the packaging cell derived human proteins. Thus immune reactions by infected host organisms may not be entirely directed against viral proteins, but also to the non-self human proteins [12]. Desthiobiotin is found in the biotin-producing plants and microbes and may make little difference in immune reactions. The potential for enhanced immune destruction of lentiviral vectors *in vivo* and/or immune reactions against transient desthiobiotinylated host cells will require investigation *in vivo*. However, avoidance of envelope modifications and targeting the lentiviral vectors via the DBL packaging cell membrane may minimise

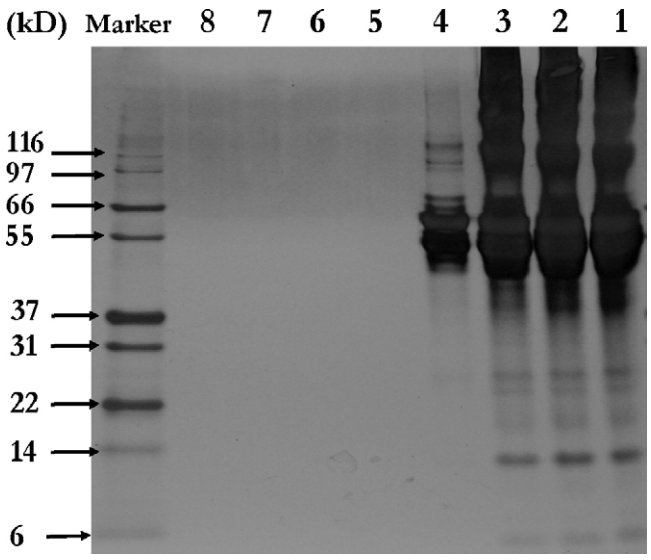

Fig. 8. SDS-PAGE analysis of 5 μL samples of the fractions from the column-based purification of desthiobiotinylated lentiviral vectors. The crude lentiviral supernatant was produced by DBL cells contacted with 2 mM 7-DAPA for 96 h, loaded and purified by adsorption to Pierce monomeric avidin coated columns followed by desorption in 2 mM biotin. Lane 1: complete DMEM for DBL cell culture. Lane 2: crude lentivirus-containing cell culture supernatant. Lane 3: lentiviral supernatant after adsorption. Lane 4: washing fraction using PBS buffer. Lanes 5–8: recovered lentiviruses in four elution fractions.

potential immunogenicity and provide a strategy that may be applicable to the capture, elution and concentration of lentiviral vectors labelled with desthiobiotin. If necessary, the residual biotin, which is present in the eluent, could be effectively removed by diafiltration, which may anyway be necessary for formulation and standardisation of titre.

4. Conclusions

Desthiobiotin-labelled lentiviruses were metabolically produced by DBL packaging cells in contact with 7-DAPA. Compared to the metabolic biotinylation technology, this novel 7-DAPA dependent desbiotinylation strategy has two major advantages which may enable scalable virus production: (1) no requirement for pre-treatments to remove free avidin-binding impurities prior to purification; (2) suitability for processes requiring efficient elution of virus from affinity supports. This desthiobiotinylation technology enables affinity-mediated paramagnetic-particle and chromatographic capture and recovery of lentiviral particles with a high purity in a highly efficient and fast way. Modifying the number of desthiobiotin molecules on the surface of lentiviral particles by varying the 7-DAPA concentration and incubation time improved the recovery of infectious viral vectors. The potential for enhanced immune destruction of lentiviral vectors *in vivo* and/or immune reactions against transient desthiobiotinylated host cells will require investigation *in vivo*. However, avoidance of envelope modifications and targeting the lentiviral vectors via the DBL packaging cell membrane may minimise potential immunogenicity. This technology provides a potential generic simple affinity recovery process for scalable production of clinical grade lentiviral vectors for gene therapy applications.

Acknowledgements

The authors wish to thank the Biotechnology and Biological Sciences Research Council (BBSRC) for funding this research. The authors are also grateful for the assistance provided by Dr Ian R. Baxendale in microwave reaction.

References

[1] B.L. Levine, L.M. Humeau, J. Boyer, R.-R. MacGregor, T. Rebello, X. Lu, G.K. Binder, V. Slepushkin, F. Lemiale, J.R. Mascola, F.D. Bushman, B. Dropulic, C.H. June, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 17372.
[2] B. Zhang, H.Q. Xia, G. Cleghorn, G. Gobe, M. West, M.Q. Wei, Gene Ther. 8 (2001) 1745.
[3] L. Pham, H. Ye, F.L. Cosset, S.J. Russell, K.W. Peng, J. Gene Med. 3 (2001) 188.
[4] J. Reiser, Gene Ther. 7 (2000) 910.
[5] M. Scherr, K. Battmer, M. Eder, S. Schüle, H. Hohenberg, A. Ganser, M. Grez, U. Blömer, Gene Ther. 9 (2002) 1708.
[6] V. Slepushkin, N. Chang, R. Cohen, Y. Gan, B. Jiang, E. Deausen, D. Berlinger, G. Binder, K. Andre, L. Humeau, B. Dropulic, Bioprocessing J. 2 (2003) 89.
[7] K. Yamada, D.M. McCarty, V.J. Madden, C.E. Walsh, Biotechniques 34 (2003) 1074.
[8] R.H. Kutner, S. Puthli, M.P. Marino, J. Reiser, BMC Biotechnol. 9 (2009) 10.
[9] R.S. O'Keefe, M.D. Johnston, N.K.H. Slater, Biotechnol. Bioeng. 62 (1999) 537.
[10] C. Summerford, R.J. Samulski, Nat. Med. 5 (1999) 587.
[11] M.M. Segura, A. Garnier, Y. Durocher, H. Coelho, A. Kamen, Biotechnol. Bioeng. 98 (2007) 789.
[12] D. Nesbeth, S.L. Williams, L. Chan, T. Brain, N.K.H. Slater, F. Farzaneh, D. Darling, Mol. Ther. 13 (2006) 814.
[13] S.L. Williams, D. Nesbeth, D.C. Darling, F. Farzaneh, N.K.H. Slater, J. Chromatogr. B 820 (2005) 111.
[14] S.L. Williams, M.E. Eccleston, N.K.H. Slater, Biotechnol. Bioeng. 89 (2005) 783.
[15] N.M. Green, Adv. Protein Chem. 29 (1975) 85.
[16] A. Wortmann, M.C. Jecklin, D. Touboul, M. Badertscher, R. Zenobi, J. Mass Spectrom. 43 (2008) 600.
[17] A.J. Otsuka, M.R. Buoncristiani, P.K. Howard, J. Flamm, C. Johnson, R. Yamamoto, K. Uchida, C. Cook, J. Ruppert, J. Matsuzaki, J. Biol. Chem. 263 (1988) 19577.
[18] V. Phalip, I. Kuhn, Y. Lemoine, J.M. Jeltsch, Gene 232 (1999) 43.
[19] J.D. Hirsch, L. Eslamizar, B.J. Filanoski, N. Malekzadeh, R.P. Haugland, J.M. Beechem, R.P. Haugland, Anal. Biochem. 308 (2002) 343.
[20] D. Savage, S. Mattson, S. Desai, G. Nielander, S. Morgensen, E. Conklin, Avidin-Binding Chemistry: A Handbook, Pierce Chemical Company, Rockford, 1992.
[21] C. Bordignon, C. Bonini, S. Verzeletti, N. Nobili, D. Maggioni, C. Traversari, R. Giavazzi, P. Servida, E. Zappone, E. Benazzi, M. Bernadi, F. Porta, G. Ferrari, F. Mavilio, S. Rossini, R.M. Blaese, F. Candotti, Hum. Gene Ther. 6 (1995) 813.
[22] P.J. Schatz, Biotechnology 11 (1993) 1138.
[23] D.F. Barker, A.M. Campbell, J. Mol. Biol. 46 (1981) 451.
[24] W.B. Tsai, M.C. Wang, Biomaterials 26 (2005) 3141.
[25] V.H.B. Ho, A. Barcza, R. Chen, K.H. Müller, N.J. Darton, N.K.H. Slater, Biomaterials 30 (2009) 6548.
[26] C. Hughes, J. Galea-Lauri, F. Farzaneh, D. Darling, Mol. Ther. 3 (2001) 623.
[27] K. Hoffmann, D.B. Melville, V. du Vigneaud, J. Biol. Chem. 141 (1941) 207.
[28] O. Livnah, E.A. Bayer, M. Wilchek, J.L. Sussman, FEBS Lett. 328 (1993) 165.
[29] T. Rodrigues, M.J. Carrondo, P.M. Alves, P.E. Cruz, J. Biotechnol. 127 (2007) 520.
[30] S. Campos, M.B. Parrott, M.A. Barry, Mol. Ther. 9 (2004) 942.