Process Biochemistry 45 (2010) 203–209

Contents lists available at ScienceDirect

Process Biochemistry

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

The harnessing of peptide–monolith constructs for single step plasmid DNA purification

Ying Han [a,*], Guanlin You [b], Leonard K. Pattenden [c], Gareth M. Forde [a]

[a] Bio Engineering Laboratory, Department of Chemical Engineering, Monash University, Vic 3800, Australia
[b] School of Engineering, University of Ballarat, Mt Helen, Vic 3350, Australia
[c] Health Innovations Research Institute, School of Medical Sciences, RMIT University, PO Box 71, Bundoora, Vic 3083, Australia

ARTICLE INFO

Article history:
Received 25 February 2009
Received in revised form 6 September 2009
Accepted 8 September 2009

Keywords:
Affinity ligands
Chromatography
DNA purification
Monolith
Plasmid DNA
Process scale up
Protein–DNA binding

ABSTRACT

The availability of synthetic peptides has paved the way for their use in tailor-made interactions with biomolecules. In this study, a 16mer LacI-based peptide was used as an affinity ligand to examine the scale up feasibility for plasmid DNA purification. First, the peptide was designed and characterized for the affinity purification of *lac*O containing plasmid DNA, to be employed as a high affinity ligand for the potential capturing of plasmid DNA in a single unit operation. It was found there were no discernible interactions with a control plasmid that did not encode the *lac*O nucleotide sequence. The dissociation equilibrium constant of the binding between the 16mer peptide and target pUC19 was $5.0 \pm 0.5 \times 10^{-8}$ M as assessed by surface plasmon resonance. This selectivity and moderated affinity indicate that the 16mer is suitable for the adsorption and chromatographic purification of plasmid DNA. The suitability of this peptide was then evaluated using a chromatography system with the 16mer peptide immobilized to a customized monolith to purify plasmid DNA, obtaining preferential purification of supercoiled pUC19. The results demonstrate the applicability of peptide–monolith supports to scale up the purification process for plasmid DNA using designed ligands via a biomimetic approach.

1. Introduction

The current high level of interest in structural biology information coupled with the growing demand for high purity DNA, employed as vaccines and gene therapies, has led to an interest in harnessing naturally occurring DNA affinity interactions for use in DNA purification and detection applications [1–9]. Furthermore, the stringent purity requirements of regulatory bodies (i.e. FDA, EMEA, TGA) [10] creates a demand for methods to obtain very pure bio-therapeutics or bio-pharmaceuticals without contaminants. This demand is particularly strong for plasmid DNA (pDNA), which has potential in the bio-pharmaceutical industry for the expression of therapeutic proteins and peptides *in vivo* and as a vaccine [5].

The production of pDNA is often accomplished by lysis of the cells, followed by a clarification/concentration step and, finally, three or more chromatography steps [11–17] to remove host cell contaminants: RNA, proteins, endotoxins and genomic DNA (gDNA), which may constitute as much as 98% of material by dry weight. Affinity chromatography can be used to specifically capture target product (pDNA) and isolate it based on specific biological function or individual chemical structure, thus having the power to reduce downstream processing when the selectivity and binding strength of optimised affinity ligands enable an otherwise multi-step purification procedure to be replaced by a single affinity purification step. However, no affinity purification protocol can be utilised without suitable ligands [18], and this is especially important to meet with the demands and rigours of industry requirements.

Several ligands for affinity purification of pDNA have been investigated at the laboratory scale. The formation of triple helices between oligonucleotides attached to a chromatographic matrix and duplex sequences (present on the plasmid) has been used to purify pDNA [19,20]. In these reports, more than 60% recovery yields were reached and the RNA and gDNA content were reduced significantly, but slow binding kinetics were displayed creating a limitation at scale [19,20]. Studies using proteins as affinity ligands to purify pDNA have also been described such as a zinc finger DNA-binding protein [21] and Lac repressor protein [22,23]. Although effective, there is a limitation of scale and economics as such ligands are large and often challenging to purify.

Despite these limitations, the application of the Lac repressor protein in displacement chromatography was shown to retain biological activity after immobilization [24]. Hasche and Voß (2005) found that interaction between repressor molecules in the form of a short operator sequence was specific for double stranded

* Corresponding author.
E-mail address: ying.han@eng.monash.edu.au (Y. Han).

1359-5113/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2009.09.008

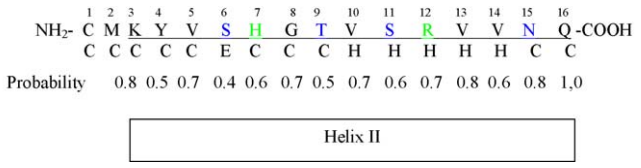

Fig. 1. Structure of the designed 16mer peptide. Termini: H–. . .–OH. The peptide comprises an N-terminal cysteine for chemical manipulation in the designer sequence and a C-terminal fragment from helix II of the Lac repressor. Secondary structure was predicted using the Advanced Protein Secondary Structure Prediction Server (APSSP) (http://imtech.res.in/raghava/apssp/) employing a nearest neighbor and neural network approach [55]. The nomenclature is in table (H = helix; e = strand; c = coil) and probability of correct assignment shown.

DNA, with no RNA interactions being detected [25]. The Lac repressor (lacI) is a DNA binding protein that regulates expression of the *lac* operon. LacI has been studied extensively at the genetic and structural level [26–33]. From the structure of the protein and DNA complex (Figs. 1 and 2, the Protein Database entry code 1JWL), the DNA binding domain consists of helix I (residues 7–13), a turn, helix II (17–26), helix III (33–47), and the hinge helix IV (50–59). Within LacI, this domain binds to DNA that is formed through association of the ~300 amino acid residue at the C-terminal domain. The binding is a complex interaction of hydrophobic interactions and hydrogen bonding [26].

Because of the well-characterised and favourable properties, LacI-based approaches have also been described for affinity adsorption of pDNA, however the yield of pDNA was low [22,23,34]. One contributing factor was the strong binding between pDNA and ligands. Ideally, the binding should be strong enough to avoid leakage during the pDNA application and wash phases, whilst enabling complete release of pDNA during the elution phase. The optimal dissociation constant for an affinity binding mechanism for use in a chromatographic system lies in the approximate range of 10^{-6} to 10^{-8} M and is the range for many affinity chromatography systems (e.g. maltodextrin–maltose binding protein (10^{-7} M), Calmodulin chromatography (10^{-8} M)) and is the target range for engineered systems where native binding affinities are too high for elution (e.g. Softlink™ avidin (10^{-7} M), *Strep*-Tactin® superflow (10^{-6} M)). This range allows designed peptides to rapidly bind with suitable affinity for pDNA, selectivity of the target pDNA without the co-purification of other biomolecules (gDNA, RNA, protein, and endotoxins), and tight binding sufficient for process rigours but appropriate for elution from the matrix.

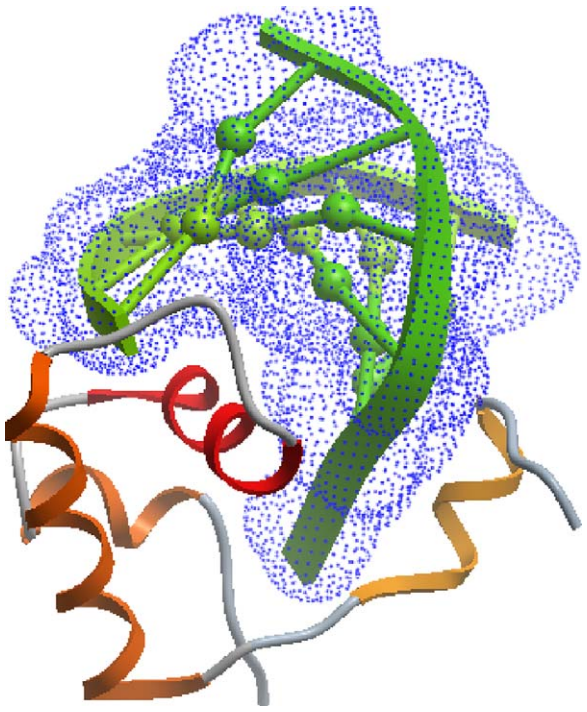

Fig. 2. The N-terminal DNA binding domain (residues 1–60) of LacI is shown with helix II present in red binding to the minor groove of the *lac*O sequence (protein data base: 1JWL is the code for the structure of the protein and DNA complex). Figure generated using Molsoft ICM Browser (Version 3.4–8f, MolSoft L.L.C.). A large contribution of bonding can be seen with the helix, which penetrates the major DNA binding groove.

For this purpose, the naturally occurring binding of *lac*O/LacI was optimized for purification of pDNA. As previously reported, synthetic peptides based on the Lac repressor protein (lacI) strongly bind pDNA containing *lac*O sequences [5]. In order to obtain *lac*O-binding peptides that bind with suitable affinity, we reasoned that deletion of some non-contributing amino acids could create a peptide that retains the DNA binding properties of the Lac repressor.

The sequence chosen for the 16mer peptide utilizes a portion of the helix II DNA binding domain of the Lac repressor (–TVSRVVNQ–) as reported from the crystal structure (Bell and Lewis, 2001). N-terminal to this fragment is a designed sequence (NH–CMKYVSHG–) which is predicted to provide a coil-like secondary structure to this region of the peptide, supportive of the natural helicity of the DNA binding domain (Figs. 1 and 2), while also providing a spacer from chromatography matrices. The cysteine residue is important for attachment to biosensor and chromatographic supports and provides known chemical manipulation such as coupling or heterobiofunctional modification.

The designed portion also imparts favourable chromatography-relevant physicochemical properties for the entire 16mer peptide [35] relevant to scaled use in chromatography such as economics and regeneration; the molecular weight is low (mw 1808.1 amu) being economical to manufacture, the p*I* is not excessively high (p*I* 9.31) as often seen for DNA binding entities which can be problematic for chemical attachment or behaviour on supports. The aliphatic index contributed by the relative volumes of the four valine residues is reasonably high (72.5%) which is regarded as a positive factor for thermostability and refolding [35,36], and the grand average of hydropathicity (GRAVY), which is a hydropathic summation of all residues, indicates a soluble peptide (−0.087) [37]. These facets favour the refolding of the peptide, which may include harsh regeneration conditions without collapse or precipitation on the surface of the column.

This study tested the utility of the designed 16mer peptide as an affinity ligand immobilized on a customized monolith to purify pUC19 pDNA containing a *lac*O nucleotide sequence. In an engineering context, the potential of process scale up is always one of primary concern. High-density bacterial lysates produced from bioreactors are very viscous and the desired pDNA are large molecules, causing significant process complexities at scale. The purification of large polynucleotides such as pDNA is hampered by the performance of conventional particulate chromatographic supports. Most of these particulate supports are made for small molecules and proteins, typically targeting molecules with diameter less than 10 nm [38]. In columns packed with such supports, large molecules such as pDNA with a size of 100 nm to over 300 nm in diameter adsorb predominantly at the beads outer surface [39]. Thus, the pore characteristics of chromatographic supports need to be considered for applications where the target molecules are several orders of magnitude larger than standard molecules typically applied to the columns. The large pores of monoliths will allow penetration of large pDNA molecules to the internal surface area at high flow rate with low pressure drops [40–45]. Polymethacrylate monolithic supports are optimal adsorbents