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Affinity analysis and application of dipeptides derived from L-tyrosine in plasmid purification

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ABSTRACT

The developments in the use of plasmid DNA (pDNA) in gene therapy and vaccines have motivated the search and improvement of optimized purification processes. In this context, dipeptides L-tyrosine-L-tyrosine and L-tyrosine-L-arginine are synthetized to explore their application as affinity ligands for supercoiled (sc) plasmid DNA (pDNA) purification. The synthesis is based on the protection of *N*-Boc-L-tyrosine, followed by condensation with L-tyrosine or L-arginine methyl esters in the presence of dicyclohexylcarbodiimide (DCC), which after hydrolysis and acidification give the afforded dipeptides. The supports are then obtained by coupling L-tyrosine, L-tyrosine-L-tyrosine and L-tyrosine-L-arginine to epoxy-activated Sepharose and are characterized by high resolution magic angle spinning (HR-MAS) NMR and Fourier transform infrared spectroscopy (FTIR). Surface plasmon resonance (SPR) biosensor is used to establish the promising ligand to be used in the chromatographic experiments and ascertain experimental conditions. Sc isoform showed the highest affinity to the dipeptides, followed by linear (ln) pDNA, being the open circular (oc) the one that promoted the lowest affinity to L-tyrosine-L-arginine. Saturation transfer difference (STD)-NMR experiments show that the interaction is mainly hydrophobic with the majority of the 5′-mononucleotides, except for 5′-GMP with L-tyrosine-L-arginine Sepharose that is mainly electrostatic. The support L-tyrosine Sepharose used in chromatographic experiments promotes the separation of native pVAX1-*LacZ* and pcDNA3-FLAG-p53 samples (oc+sc) by decreasing the salt concentration. The results suggest that it is possible to purify different plasmids with the L-tyrosine Sepharose, with slight adjustments in the gradient conditions.

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1. Introduction

The development of nucleic acid-based therapies, such as gene therapy and DNA vaccination, which require large quantity and high purity of these molecules led to the improvement of DNA purification processes, namely plasmid DNA (pDNA) purification [1]. The super-coiled (sc) pDNA recovery and purification from a lysate is extremely important due to the effectiveness of this isoform in the transfection experiments and in the subsequent expression of the target gene [2]. For this purpose, affinity chromatography has been widely used to isolate the sc plasmid isoform in a single chromatographic step, whereas RNA, gDNA, proteins and endotoxins are efficiently eliminated [3–5]. Exploiting the multiple non-covalent interactions involved in affinity chromatography it is possible to induce a selective biorecognition of the target biomolecules relying on a strong but reversible interaction between the pDNA and the immobilized ligand of the matrix [6].

Amino acids have been used as affinity ligands since they act as electron acceptors, have high physical and chemical stability and are easy to be obtained in large quantities at a relatively low cost [7]. Numerous amino acid have been recently employed as ligands in affinity chromatography matrices in order to purify sc pDNA such as L-histidine and L-arginine, used to purify sc pDNA from a clarified *Escherichia coli* lysate and fully separate sc pDNA from oc isoform [5–8]. The underlying mechanisms of biorecognition and interactions were also studied by techniques such as surface plasmon resonance (SPR) and nuclear magnetic resonance (NMR) [9,10]. The dipeptides have been poorly investigated as affinity ligands for purification of plasmid DNA. This can be attributed to the low availability of dipeptides due to the lack of an efficient and cost-effective process for dipeptide production [11]. Dipeptide syn-

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thesis can be accomplished either by using chemical or enzymatic methods [12].

Based on this, other amino acids such as L-tyrosine and its dipeptide derivatives are potential and versatile affinity ligands for sc plasmid purification. The polar nature of L-tyrosine favours the interaction with the DNA through amphipathic interactions (imino group hydrogen bonding, specific dipolar interactions, cation-π and hydrophobic interactions) [13]. Moreover, L-tyrosine has been described to interact with DNA through intercalative modes [14]. The current study focus on development of new supports based on dipeptides derived from L-tyrosine, L-tyrosine-L-tyrosine and L-tyrosine- L-arginine (see Fig. 1), followed by the screening of their affinity to pVAX1-*LacZ* and pcDNA3-FLAG-p53 isoforms using SPR-biosensor and amino acid-nucleotide interactions using STD-NMR spectroscopy. Finally, the retention behaviour of pVAX1-*LacZ* and pcDNA3-FLAG-p53 was also evaluated by chromatography using the L-tyrosine support.

2. Material and methods

All the water used to prepare solutions was ultra-pure grade, purified with a Milli-Q system from Millipore. L-tyrosine and L-arginine methyl ester were purchased from Sigma–Aldrich. NZY Maxi Prep Kit was purchased from NZYTech (Lisbon, Portugal). The 6.07 kbp pcDNA3-FLAG-p53 addgene plasmid 10838 [15] was from Addgene (Cambridge, MA, USA), the 6.05 kpb pVAX1-*LacZ* was purchased to Invitrogen (Carsland, CA, USA) and all the reagents used in bacterial growth were obtained from Sigma–Aldrich. The DNA ladder was obtained from Bioline (London, UK).

2.1. Synthesis of dipeptides L-tyrosine-L-tyrosine and L-tyrosine-L-arginine

The *N*-tertbutoxycarbonyltyrosine and L-tyrosine methyl ester were synthesized according to following procedures.

2.1.1. L-tyrosine methyl ester

Thionyl chloride (0.15 mol, 1.14 mL) was added dropwise to methanol (10 mL) at −10 °C whilst stirring. The mixture was stirred for a further 0.5 h at −10 °C. L-tyrosine (0.013 mol, 2.4 g) was added to the reaction solution and stirred for 1 h at −10 °C. The ice bath was removed and the reaction mixture was allowed to stir for 2 h. The solution was heated to reflux at 50 °C for 30 min. After that the mixture was concentrated in vacuum to half of the volume and precipitated with 10 mL of ethyl acetate. The precipitate was recrystallized with ethyl acetate and dried at 55 °C for 24 h to obtain the pure compound as a white solid.

Yield: 97%

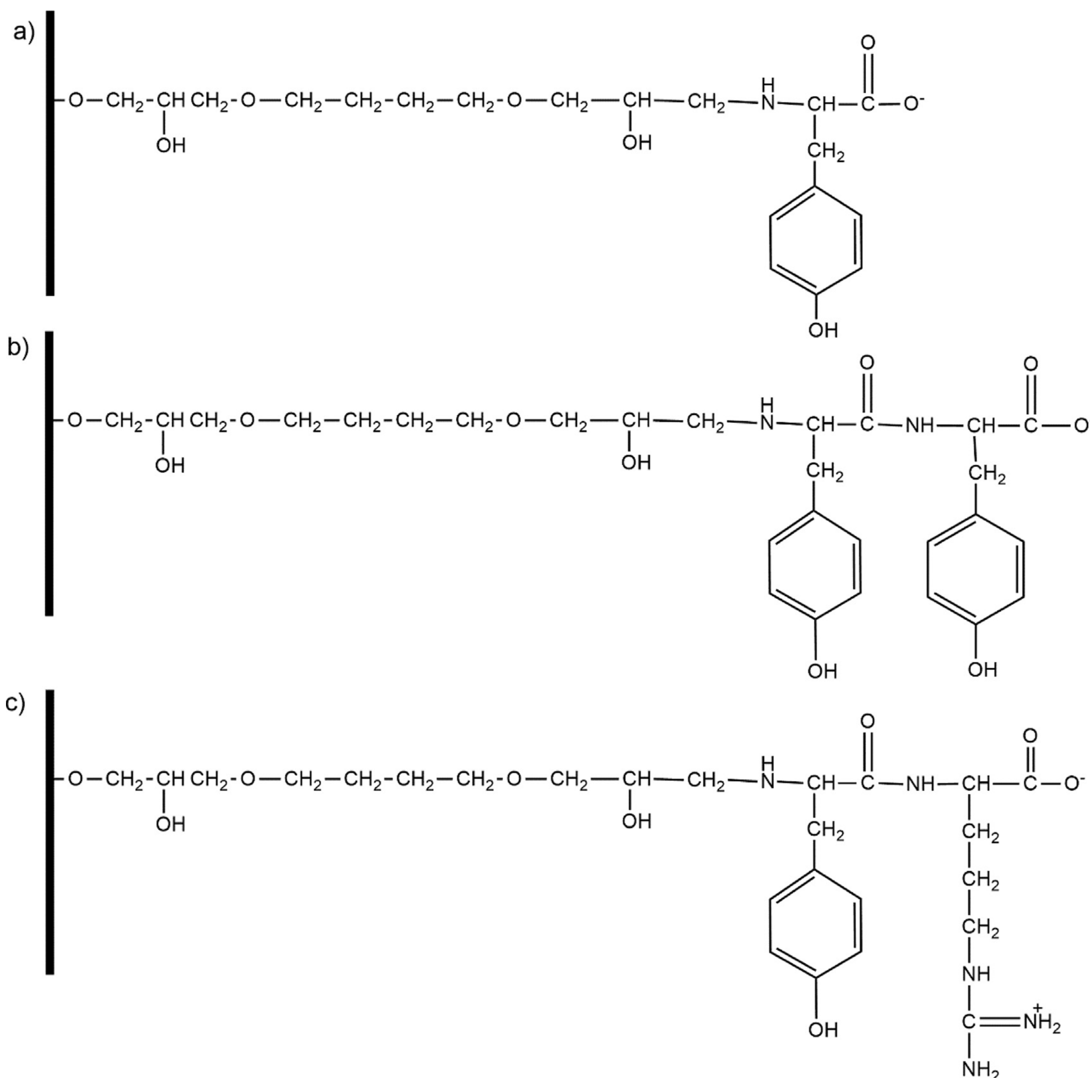

Fig. 1. Chemical structures of the supports (a) L-tyrosine Sepharose (b) L-tyrosine-L-tyrosine Sepharose and (c) L-tyrosine- L-arginine Sepharose.

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