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Thermodynamic study of the interaction between linear plasmid deoxyribonucleic acid and an anion exchange support under linear and overloaded conditions

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ABSTRACT

Anion-exchange chromatography has been successfully used in plasmid DNA (pDNA) purification. However, pDNA adsorption mechanism using this method is still not completely understood, and the prediction of the separation behavior is generally unreliable. Flow microcalorimetry (FMC) has proven its ability to provide an improved understanding of the driving forces and mechanisms involved in the adsorption process of biomolecules onto several chromatographic systems. Thus, using FMC, this study aims to understand the adsorption mechanism of linear pDNA (pVAX1-*LacZ*) onto the anion-exchange support Fast Flow (FF) Q-Sepharose. Static binding capacity studies have shown that the mechanism of pDNA adsorption onto Q-Sepharose follows a Langmuir isotherm. FMC experiments resulted in thermograms that comprised endothermic and exothermic heats. Endothermic heat major contributor was suggested to be the desolvation process. Exothermic heats were related to the interaction between pDNA and Q-Sepharose primary and secondary adsorption. Furthermore, FMC revealed that the overall adsorption process is exothermic, as expected for an anion-exchange interaction. Nevertheless, there are evidences of the presence of nonspecific effects, such as reorientation and electrostatic repulsive forces.

1. Introduction

The potential of pDNA as a therapeutic molecule in the treatment and cure of several diseases, through DNA vaccines or gene therapy, has been sustained by innumerous recent studies [1–4]. Plasmid-based therapies require efforts from researchers to develop cost-effective technologies to produce large quantities of highly pure pDNA [5]. Anion-exchange chromatography has been successfully used in pDNA purification [1,4,6–8], however its mechanism of interaction is still not completely understood. Additionally, in industry, due to economic reasons, it is usual to perform chromatographic runs in non-linear conditions, through feed overloading in concentration or volume [9]. Under these conditions adsorption mechanism complexity is further increased.

Thermodynamic parameters related to adsorption and desorption of biomolecules onto chromatographic media have helped to elucidate complex adsorption mechanisms in liquid chromatography. These parameters can be accessed from microcalorimetric measurements, by analyzing data through van't Hoff plots or by performing batch equilibrium experiments [10–14]. Nevertheless, these methods may not produce representative results of overloaded conditions, batch equilibrium experiments have limited resolution and the indirect method of van't Hoff analysis may be complicated by the presence of multiple adsorption sub-processes. Conversely, calorimetric methods such as Isothermal Titration Microcalorimetry (ITM) and flow microcalorimetry (FMC) have shown their aptitude to understand the underlying adsorption mechanism of several proteins in several chromatographic media, including linear and overloaded conditions [13–17].

Despite the progress in the understanding of adsorption mechanism for proteins through thermodynamic studies [11,14–20], there is still a lack of information for plasmids adsorption onto chromatographic supports [7]. Few studies have addressed this issue. Ferreira et al. [7] studied the batch adsorption of pDNA onto anion-exchange chromatographic supports and concluded that is plausible to apply the Langmuir isotherm model. The Langmuir isotherm [21] is the simplest theoretical model usually applied to

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describe biomolecules adsorption onto ion exchangers, however, the data obtained does not account for non-ideal effects neither the complexity of working in overloaded conditions. Tarmann and Jungbauer [22] have used van't Hoff analysis to investigate DNA retention on Source 30 Q at different temperatures and salt concentrations. It was shown that the adsorption process was driven entropically in all studied cases. More recently, Mahut et al. [23] reach the same conclusion when reporting on the separation of pDNA isoforms on silica-based columns modified with a quinine carbamate ligand. The observed behavior was related with changes in the solvation shell (and possibly the twist of the DNA helix) upon the binding to the stationary phase. Chen et al. [24] performed a study of the interaction mechanism between single-strand (ss) and double-strand (ds) DNA with hydroxyapatite (HA) by Isothermal Titration Microcalorimetry (ITM) and static binding measurements. ITM revealed that the dehydration is the dominant step in the interaction process. Furthermore, it was concluded that the binding behavior between dsDNA and HA is mainly driven by electrostatic interactions and ssDNA binding is more complex due to the hydrophobic and π–π interaction between bases. Lastly, Phillips and Pinto [25], using flow microcalorimetry (FMC), performed a study that closely resembles the adsorption of DNA. They investigated the adsorption of nitrogen bases and nucleosides onto a hydrophobic interaction adsorbent. FMC simulates a chromatographic system in its operation mode, thus it is expected that its results may be representative of what happens in an actual chromatographic column [14]. Phillips and Pinto [25] study revealed that the adsorption behavior of nitrogen bases and nucleosides is a complex phenomenon highly dependent on the type of molecule. Furthermore, although hydrophobic interaction appears to be the primary mechanism for the adsorption, they observed that the measured heats of adsorption resulted from the net effect of two different types of interactions: adsorbate/adsorbent interactions and base stacking self-interactions between like molecules.

Considering the already proven ability of flow microcalorimetry (FMC) in understanding the driving forces and mechanisms of biomolecules adsorption onto several chromatographic supports [14–20,26–29], the present study used FMC as a central technique to understand the adsorption mechanism of linear (ln) pDNA (pVAX1-*LacZ*) onto the anion-exchange support FF Q-Sepharose, considering linear and overloaded conditions.

2. Experimental methods

2.1. Plasmid production, recovery and purification

The 6.05 kbp plasmid, pVAX1-*LacZ* (Invitrogen, Carlsband, CA, USA), was obtained by *Escherichia coli* (*E. coli*) DH5α fermentation as described by Sousa and Queiroz [30]. The plasmid was recovered from the cells and purified using the QIAGEN® Plasmid Maxi Kit. Isolated pDNA was analyzed by horizontal electrophoresis according to Caramelo-Nunes et al. [31]. Linear pDNA isoform was obtained using the restriction endonuclease Hind III (NZYTech, Lisbon, Portugal). Hind III was removed from pDNA samples through centrifugation, using 100 kDa Vivaspin membrane concentrators (Sartorius Stedim Biotech, Madrid). For storage and as a carrier fluid 10 mM Tris–HCl (Sigma–Aldrich, Madrid, Spain), pH 8.0 was used.

2.2. Adsorption isotherm measurements

Adsorption isotherms were performed in multiwell plates, where 10 mg of dry FF Q-Sepharose have been placed in each well, adding 1 mL of a known concentration of ln pDNA in 10 mM Tris–HCl, pH 8.0, as described by Silva et al. [14]. After 24 h [7] equilibrium reached and pDNA content of mobile phase was determined by spectrophotometry (UV, 260 nm). All experiments were performed in triplicates. By fitting experimental data to the Langmuir model of adsorption, maximal binding capacities q_{max} and association constant K_A were determined. The general equation for the Langmuir isotherm is:

$$q^* = \frac{q_{max} K_A C^*}{1 + K_A C^*} \quad (1)$$

where C^* is the biomolecule equilibrium concentration in solution, q^* is the biomolecule solid-phase equilibrium concentration, q_{max} is the maximum adsorption capacity of the adsorbent and K_A is the adsorption constant.

2.3. Flow microcalorimetry (FMC)

Thermodynamic studies were performed in the flow microcalorimeter (Microscal FMC 4 Vi, Microscal Limited, London, UK). The flow microcalorimeter has the ability to measure the heat flow caused by interaction during the adsorption process of biomolecules onto chromatographic media. Interfaced with its cell, the microcalorimeter has two highly sensitive thermistors that are capable of detecting small temperature changes. The heat evolution or absorption during an interaction is indicated by changes in potential (imbalance in the thermistor bridge in which the two thermistors measure temperature changes in the cell). Thus, when an exothermic interaction occurs the microcalorimeter will sense an increase in energy and a positive signal will appear in the thermogram. The opposite is observed for an endothermic interaction. Experiments were performed as described by Silva et al. [14]. The system was packed with approximately 21.9 mg of dried Q-Sepharose to fill the 171 μL cell. After thermal equilibrium attainment, by passing the equilibration buffer (10 mM Tris–HCl, pH 8.0) through the cell at a constant flow rate of 1.5 mL/h for 12 h, a sample of ln pDNA (in 10 mM Tris–HCl, pH 8.0) was loaded into a configurable injection loop (30, 229 or 429 μL) and then injected into the cell using a constant flow rate of 1.5 mL/h. Between injections, 1 M NaOH was used as a washing solution.

CALDOS 4 software (Microscal Limited, London, UK) was used to acquire, store, and process all the FMC data. Peak deconvolution was performed by the PEAKFIT software package (version 4.12, Seasolve Software Inc., San Jose, USA) using asymmetric Gaussian peaks.

3. Results and discussion

The topological linear pDNA form used in this study was prepared enzymatically. High-resolution agarose gel electrophoresis was used to track the production process (Fig. 1). The use of the Qiagen Plasmid Maxi kit® yielded approximately a final concentration of 600 $\mu g\ mL^{-1}$ of 6.05 kbp pVAX1-lacZ pDNA (linear, open circular and supercoiled isoforms) (Fig. 1[A]). Subsequently, the pDNA was left at room temperature for three days. During this time span, an increase in the relative amount of open circular and linear pDNA and a parallel decrease in the relative amount of supercoiled pDNA was observed. In the third day, only circular and linear pDNA were present (Fig. 1[D]-(a)). After digestion with the restriction enzyme Hind III pure linear pDNA was obtained (Fig. 1[D]-(b)).

Static binding capacity results of ln pDNA adsorption onto Q-Sepharose are reported in Fig. 2. Through the analysis of the equilibrium isotherm profile, it can be seen that the adsorbed ln pDNA concentration (q_{pDNA}) increases from zero to a plateau region as the ln pDNA equilibrium liquid concentration (C_{pDNA}) increases. Within the range covered in this study, the shape of the curve fits to the Langmuir (type I) isotherm profile [12,21]. Langmuir fitted parameters, association constant (K_A) and maximum binding capacity (q_{max}) were respectively 0.31 ± 0.04 mL μg^{-1} and