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Thermodynamics of the adsorption of monoclonal antibodies in phenylboronate chromatography: Affinity *versus* multimodal interactions

Sara A.S.L. Rosa [a], C.L. da Silva [a,b], M. Raquel Aires-Barros [a], A.C. Dias-Cabral [c,d,**], Ana M. Azevedo [a,*]

[a] *iBB- Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*
[b] *The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal*
[c] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal*
[d] *Department of Chemistry, University of Beira Interior, Covilhã, Portugal*

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ABSTRACT

The aim of this work was to investigate the complex phenomena underlying the adsorption of an anti-human IL-8 (anti-IL8) monoclonal antibody (mAb) to *m*-aminophenylboronate (*m*-APBA) by Flow Microcalorimetry (FMC) and to understand the role of non-specific interactions in the adsorption process. FMC was exploited as a dynamic *on-line* method to measure instantaneous heat energy transfers in order to understand the surface phenomena underlying mAb's adsorption towards the synthetic ligand *m*-APBA under different pH (7.5, 8.5, 9.0, 9.5 and 10.0) and salt concentrations (0 and 150 mM NaCl). Results showed that the adsorption of anti-IL8 mAb to *m*-APBA is enthalpically driven (ΔH_{ads}<0), as expected for the predominant reversible esterification reaction between boronates and *cis*-diols-containing molecules. For all the pH conditions studied, thermograms presented a first exothermic peak, characteristic of the reversible esterification reaction between mAb (pI≥9.3) and *m*-APBA (pKa = 8.8), except at pH 9.0 in the presence of 150 mM NaCl, for which the thermogram presented a first endothermic peak. The heat of adsorption (ΔH_{ads}) obtained at conditions where *cis*-diol interactions were predominant was approximately -243 ± 38 kJ/mol against -82 ± 14 kJ/mol (p-value < 0.05) obtained at pH 9.0 with 150 mM NaCl. The observed shift in the thermogram profile at pH 9.0, 150 mM NaCl, and the consequent decrease of 60–70% in ΔH_{ads} were indicative of the promotion of electrostatic interactions between the protein and the ligand. Overall, and whereas the binding of the PBA ligand to mAb molecules has been described for decades as being affinity-based, our study demonstrates the multimodal behaviour of this interaction and contributes towards the understanding of the adsorption thermodynamics.

1. Introduction

Therapeutic products based on monoclonal antibodies (mAbs) still represent the major class of biopharmaceutical products with worldwide sales expected to reach $125 billion by 2020 [1]. This continuous trend has been related to major technology developments in the upstream processing [2–4]. The increasing knowledge of global authorities on the production process together with the effectiveness and safety of these therapeutic products have been the major driving forces for such success.

However, despite all efforts, a more generalized access to these biopharmaceuticals is still barred by high selling prices, with downstream processing being considered the bottleneck in the manufacturing of mAbs. The established downstream processing relies on a platform-based approach that encompasses a protein A affinity chromatography capture step, which can represent up to 25% of the total manufacturing costs [5]. Therefore, the design of novel and more cost-effective operations and their implementation in the current industrial settings represents a major need. The alternatives range from non-chromatographic techniques, like aqueous two-phase separation, membrane filtration, precipitation or crystallization, to affinity chromatographic steps not based on protein A and emergent strategies like multimodal chromatography [6,7].

* Corresponding author at: Avenida Rovisco Pais, 1, 1049-001 Lisboa, Portugal.
** Corresponding author at: Avenida Infante D. Henrique, 6200-506 Covilhã, Portugal.
E-mail addresses: ccabral@fcsaude.ubi.pt (A.C. Dias-Cabral), a.azevedo@tecnico.ulisboa.pt (A.M. Azevedo).

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Phenylboronic acid (PBA) is a synthetic ligand that has been widely used in affinity chromatography for the specific capture and effective enrichment of a large diversity of target *cis*-diol-containing molecules, such as carbohydrates, glycoproteins, RNA, nucleotides and nucleic acids [8–12]. Its affinity relies on the ability of the boronate ligand to establish a pair of covalent bonds with molecules containing *cis*-diols *via* a reversible esterification reaction [13]. PBA can bind to antibodies *via* the oligosaccharides N-linked at the Asn^{297} of the CH2 domain of the Fc region of each heavy chain, which are typically composed of 1,2- *cis*-diol saccharides such as fucose, mannose and galactose [14–16].

In acidic to neutral environments (pH < pKa), boronic acids adopt a trigonal planar form, which can originate a tetrahedral boronate anion upon hydroxylation in alkaline conditions (pH > pKa). Both the acid and its conjugate base can bind to diol compounds [17]. However, since the equilibrium constant for the tetrahedral (K_{tet}) form is usually higher compared to the trigonal one (K_{trig}), complexes are prone to be less stable under acidic conditions [18].

Nonetheless, considering the complex nature of PBA ligands, adsorption can also be induced by non-specific interactions. For example, PBA ligands can also establish hydrophobic and π-π interactions due to their aromatic nature. Secondary ionic interactions between boronates and charged molecules can also occur through coulombic attraction or repulsion effects, which means that PBA may act as a weak cation exchanger [19,20]. Hydrogen bonding and charge transfer interactions are also possible. The latter is more likely to take place under acidic conditions since in the trigonal uncharged form the boron atom has an empty orbital and, thus, can serve as an electron acceptor. This will enable Lewis acid-base interactions to occur with negatively charged carboxylated groups (*e.g.*, from aspartate and glutamate residues) or unprotonated amino groups (*e.g.*, from asparagine and glutamine residues) of proteins [9,21].

Given the range of interactions described above, PBA has been classified by many authors as a multimodal ligand [8,22]. Therefore, the challenge lies in understanding and consequently predicting the adsorption behaviour of proteins, including mAbs, onto PBA-chromatographic matrices. In order to depict the complex phenomena involved, the evaluation of the interaction between solutes and the chromatographic matrices is critical. The knowledge acquired in this context will contribute towards a better understanding of the process, essential to ensure process consistency under the quality attributes established by the Quality by Design (QbD) initiative [23]. Therefore, a deeper understanding of these phenomena, combined with the economic advantages of employing the PBA ligand [24], could lead the industry to adopt such a chromatographic process as a reliable alternative to protein A-based capture steps.

Techniques like isothermal titration calorimetry (ITC) [25–28], confocal laser scanning microscopy [29], nuclear magnetic resonance [30], and surface plasma resonance [31] have been used to study ligand-protein molecular interactions. However, none of them can account for the dynamics of the chromatographic process. This restricts the understanding of the adsorption mechanism and thus the optimization of ligand design and overall process. Flow microcalorimetry (FMC) has proven to be an effective approach to obtain *on-line* and *in-situ* heat signal measurements of adsorption and desorption events occurring in different chromatographic systems [32–34]. A flow microcalorimeter has the ability to simulate a packed-bed chromatographic system at micro-scale, where the profile obtained provides an overview on the kinetics of the adsorption and desorption of biomolecules such as proteins [35–37] and pDNA [38].

The aim of this work was to understand the complex phenomena underlying the adsorption of a full anti-human IL-8 mAb (pI≥9.3) obtained from a clarified supernatant of Chinese Hamster Ovary (CHO) cell cultures, towards the synthetic *m*-aminophenylboronic acid (*m*-APBA) ligand (pKa = 8.8). To this end, FMC was exploited as an emergent tool for thermodynamic *in-situ* and *on-line* evaluation of the mechanisms associated with the adsorption of an IL8-mAb in boronate chromatography, as well as to elucidate the role of non-specific interactions during the adsorptive process. Considering the ligand structure and possible conformations of *m*-APBA, studies were performed under different pH (7.5, 8.5, 9.0, 9.5 and 10.0) and salt concentrations (0 and 150 mM NaCl).

2. Material and methods

2.1. Chemicals

Tris(hydroxylmethyl)aminomethane (Tris), sodium citrate monobasic, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonicacid (EPPS) and 2-(cyclohexylamino)ethanesulfonic acid (CHES) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 4-(2-hydroxyethyl)-1-piperazineethanesulfonicacid (HEPES), sodium chloride (NaCl) and hydrochloric acid were obtained from Fisher Scientific (Waltham, MA, USA). Sodium phosphate monobasic anhydrous and sodium phosphate dibasic were acquired from Acros Organics (Geel, Belgium) and sodium azide (NaN_3) from Panreac (Barcelona, Spain). Sodium hydroxide (NaOH) was purchased from José Manuel Gomes dos Santos Lda. (Odivelas, Portugal). All other chemicals were analytical or HPLC grade. Water used in all experiments was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Production, purification, concentration and diafiltration of anti-IL8 mAb

An anti-human interleukin-8 (anti-IL8) mAb, with an isoelectric point around 9.3, was produced by CHO cells under serum-free culture conditions and purified using protein A affinity chromatography. After purification, the anti-IL8 mAb was concentrated and diafiltered using the different adsorption buffers studied. The final concentration of mAb solution was 5 mg/mL. Further details on these procedures are provided as Supplementary material (Section S1).

2.3. Flow Microcalorimetry (FMC)

Thermodynamic studies were performed in a flow microcalorimeter (Microscal FMC 4 Vi, Microscal Limited, London, UK), operated in heat conduction mode. The 171 μL microcalorimeter cell is interfaced with two highly sensitive thermistors capable of detecting power changes with a magnitude of 10^{-7} W, resulting in an energy resolution in the order of 10^{-9} J. The flow-rate through the cell is controlled by precision syringe pumps (Harvard Apparatus, UK). A block heater is used to monitor and control the cell temperature. The FMC is also equipped with a multiport valve and an automated injection system, as with a conductivity monitor and a UV detector from Pharmacia (Uppsala, Sweden).

Data acquisition, storage and processing were achieved using CALDOS 4 software (Microscal, Limited, UK). The calibration factor was obtained from the correlation between the areas of the peaks and the energy of heat pulses (3 mJ) resulting from electrical impulses of known power and duration. Peak de-convolution and peak area determination were performed with PeakFit 4.12 software (Seasolve Software Inc., San Jose, USA) using the Exponentially Modified Gaussian (EMG) model. The latter was employed due to its ability to model asymmetric signals. The exothermic and endothermic contributions to the overall heat of adsorption were calculated from the area of the respective de-convoluted peaks.

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