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Static and dynamic binding behavior of an IgG2 monoclonal antibody with several new mixed mode affinity adsorbents

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ABSTRACT

The binding behavior of several mixed-mode chromatographic adsorbents, derived from different terpyridine-based ligands immobilized onto Sepharose FF™, has been investigated with a humanized IgG2 monoclonal antibody. Static adsorption isotherms were determined and the derived parameters used to guide the choice of ligand structure and adsorption conditions to achieve favorable IgG2 mAb binding under dynamic loading conditions. The binding and elution behavior of selected adsorbents in packed chromatographic columns were studied with the purified IgG2 mAb and crude cell culture broths containing the same IgG2 mAb. Clearance of host cell proteins was found to be strongly influenced by the structure of the ligand used to generate these mixed mode resins. One ligand candidate in particular, ES-(Cl.S.Cl)terpy, was found to possess selectivity on a par with the traditionally employed Protein A affinity adsorbents for the purification of monoclonal IgG2s with an excellent level of clearance of host cell proteins. Moreover, high binding capacities, *e.g.* between 34 and 70 mg IgG2 mAb/mL resin, were achieved with these new adsorbents.

1. Introduction

The production of monoclonal antibodies (mAbs) by murine hybridoma cells in culture was discovered in the mid 1970s by Kohler and Milstein [1–3]. Since then, mAbs have found a plethora of applications, ranging from diagnostic methods to biopharmaceuticals for the effective treatment of numerous medical disorders, including cancers and autoimmune diseases [4–6]. Target specificity is the prime characteristic of mAbs, which propels their ongoing popularity [6]. The biotechnology industry is now able to provide highly productive upstream cell culturing capabilities that allow large amount of a mAb to be expressed in a consistent and efficient fashion [7,8]. However, downstream processing remains a bottleneck [9,10], which translates into higher manufacturing costs and lower productivities [11,12], with up to 60% of the entire costs associated with mAb production reportedly incurred at the downstream processing stages. Achievement of high levels of purification of mAbs and other recombinant proteins from complex cell culture broths in a cost efficient manner thus represents some of the more challenging tasks remaining in modern biotechnology [13,14].

Current research and industry practice for the primary capture of monoclonal antibodies from crude cell culture feedstocks frequently involves the use of a Protein A affinity chromatographic step [15]. Several drawbacks, including the relatively high cost and chemical instability of Protein A under the high pH conditions used during cleaning-in-place (CIP) protocols, have motivated the research community to search for substitutes [16,17]. Amongst these alternatives, synthetic ligands have recently garnered an increased level of interest as they can potentially act as substitutes for protein-based affinity ligands, including the more stable genetically engineered Z-domain mutants of Protein A [18,19], due to their chemical robustness, diversity of structure and opportunity for novel target design [17,20,21]. A further driver for the use of alternative adsorbents as substitutions for the Protein A affinity chromatographic step, which can represent up to 35% of the total raw material costs in mAb purification processes, is their potential to achieve a higher productivity and to require fewer items of process equipment, thus enabling cost reductions [22,23]. Successful market examples of the use at the laboratory and process scales

Abbreviations: c^*, equilibrium binding concentration; c_i, initial protein concentration; HCP, host cell protein; K_a, equilibrium binding constant; mAb, monoclonal antibody; MEP, 4-mercaptoethylpyridine; PSEAs, pyridinylsulfanylethylamines; v, ratio of the volume of adsorbent to total volume of incubate; q^*, amount of protein per mL adsorbent bound at equilibrium; q_m, maximum binding capacity; terpy, 4′-aminoethylsulfanyl-2,2′:6′,2″-terpyridine; TIG, terpy-immobilized gels.

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of adsorbents derived from synthetic ligands have included several triazine dye ligands as Protein A biomimetics and 4-mercaptoethylpyridine (MEP) based ligands [16,24–28]. In the latter case, the chemical attributes of MEP-based adsorbents enable protonation/deprotonation of the immobilized ligand to occur when the buffer pH is changed, permitting them to operate under thiophilic and hydrophobic charge induction chromatography (HCIC) conditions [29]. High concentrations of a kosmotropic salt together with neutral or mildly alkaline buffers enable the immobilized heterocyclic π-electron-rich ring found within the MEP ligand or structurally related ligands, such as 2-mercaptothiazole [30], to interact with proteins and thus retain them on the resin. To achieve protein desorption, mildly acidic conditions can be employed, leading to protonation of the ligand's heteroatom(s) and the bound protein(s), creating mutual electrostatic repulsion. Utilization of adsorbents with such multiple molecular mechanism characteristics in protein purification is frequently referred to, in a general context, as mixed mode separations [31,32].

As an extension to the use of thiophilic adsorbents, recently we reported the synthesis and use of a family of substituted pyridinylsulfanylethylamines (PSEAs), which when immobilized onto Sepharose 6 FF™ offered a new class of mixed mode adsorbents that could be used to purify a range of recombinant proteins, including mAbs, cytokines and transferrins [33–36]. During investigations related to the development of a PSEA analog library, a 4′-aminoethylsulfanyl-2,2′:6′,2″-terpyridine (terpy) ligand was also synthesized [33]. In preliminary studies, this ligand when immobilized onto activated Sepharose 6 FF™ gels showed [37] promising capabilities for the purification of humanized monoclonal antibodies of various different isotypes, thus potentially offering an alternative to Protein A affinity chromatography. In addition, its metal chelating properties resulted in its application in immobilized metal ion affinity separation of histidine-tagged proteins [37]. Consequently, we have extended our synthetic efforts to generate additional terpyridine-(terpy)-based ligands with substituted heterocyclic ring structures to explore their potential in mAb purification.

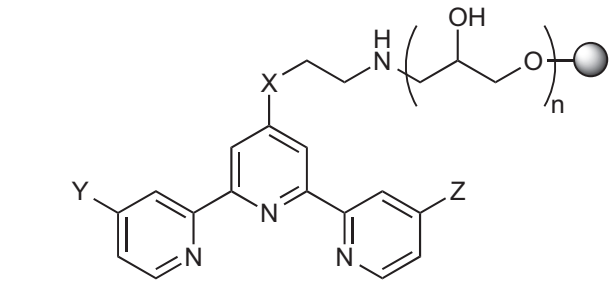
Herein, we report the mixed mode behavior of several exemplars of these substituted terpy ligands, immobilized onto Sepharose 6 FF™ (the so-called TIG resins, Fig. 1) for the purification of humanized monoclonal IgGs. In these cases, the ligand structures differ from each other at the 4,4″-positions due to substitution with the functional groups R = Cl, H, Me, and OMe, respectively. Static and dynamic binding studies have been carried out with a humanized IgG2 mAb. The results demonstrated that these functional group substitutions to the terpy core structure of the ligand have a profound influence on the selectivity with regard to host cell protein clearance, as well as the binding and elution behaviors of the IgG2 mAb with these adsorbents.

2. Materials and methods

2.1. Materials

2.1.1. Monoclonal antibodies

The antibody samples used in all experiments were prepared either from a monoclonal IgG2 solution (6.7 mg/mL in sodium acetate buffer pH 5.0, 220 mM NaCl) obtained from the purification of this humanized mAb from the harvest of a genetically engineered CHO cell line cultured in chemically defined media using Protein A affinity chromatography as reported previously [33–36], or directly from the cell culture broth containing the same IgG2 mAb (2.3 mg/mL). These IgG2 mAb samples, proprietary in nature, were generously provided by our external industrial collaborators.



	Ligand density (μmol/g)
TIG #1: X=S; Y,Z=H; n=1	296
TIG #2: X=S; Y,Z=H; n=3	285
TIG #3: X=O; Y,Z=H; n=1	285
TIG #4: X=S; Y,Z=Me; n=1	156
TIG #5: X=S; Y,Z=MeO; n=1	234
TIG #6: X=S; Y=MeO; Z=Cl; n=1	282
TIG #7: X=S; Y,Z=Cl; n=1	284
TIG #8: see below structure	259

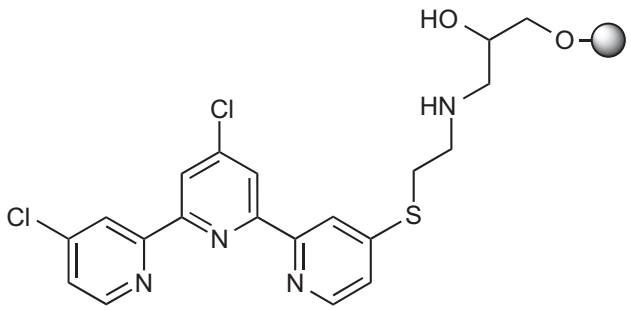


Fig. 1. Chemical structures of the terpyridine-immobilized gels (TIGs). All terpy ligands were immobilized onto epichlorohydrin-activated Sepharose 6FF gels *via* a glycidyl group (ES; $n = 1$) or a glycidyl diglycerol group (EGS; $n = 3$). Ligand densities are reported as μmol per gram of freeze-dried gel. The resin code is as follows: TIG #1: ES-terpy; TIG #2: EGS-terpy; TIG #3: ES-Oterpy; TIG #4: ES-(Me.S.Me)terpy; TIG #5: ES-(MeO.S.OMe)terpy; TIG #6: ES-(MeO.S.Cl)terpy; TIG #7: ES-(Cl.S.Cl) terpy; TIG #8: ES-(Cl.Cl.S)terpy.

2.1.2. Reagents

The analytical grade reagents Tris, Tris–HCl, MES, sodium sulfate, sodium chloride, sodium acetate trihydrate, SDS, ethanol, methanol, and glycine, were purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). Solutions of the relevant buffers were prepared using Milli-Q® (Millipore, Bedford, MA) purified water.

2.1.3. Preparation of the different Sepharose 6 FF™ adsorbents containing the immobilized terpyridine ligands (TIGs)

The synthesis of the various terpy ligands (Fig. 1) and the preparation of corresponding adsorbents were based on procedures described previously [33–37]. The ligand densities were determined by elemental analyses at the Campbell Microanalytical Laboratory, Department of Chemistry, University of Otago, Dunedin, New Zealand, and the results expressed as μmol/g adsorbent (see table within Fig. 1).

2.2. Batch and dynamic binding methods

The choice of the loading buffers took into consideration conditions previously optimized [33–37] for the pyridinylsulfanylethylamine (PSEA) ligands, including the parent terpyridine ligand, and were re-evaluated using the Tecan robotic system, whilst the choice of the optimum elution buffer was based on the results reported by Zhang et al. [38] for 4′-terpyridinylsulfanylethylamine based resins for mAb purification.