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Application of 4′-terpyridinylsulfanylethylamine resins for the purification of monoclonal antibodies by mixed-mode chromatography

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ABSTRACT

In this study, a pyridine-based compound, 4′-terpyridinylsulfanylethylamine (4′-TerPSEA), has been employed as a ligand to purify *via* mixed-mode chromatographic procedures a humanised monoclonal antibody of the IgG_1 sub-class directly from crude supernatants derived from cultured CHO cells. The antibody binding capacity, selectivity and reusability of the adsorbent, derived from the immobilisation of this ligand onto Sepharose FF™, were compared to a Protein A affinity resin. The chromatographic performance of this mixed mode adsorbent was similar to that shown by the Protein A-based adsorbent with this IgG_1 mAb. In addition, the IgG_1 mAb was able to bind to the immobilised 4′-TerPSEA under reducing conditions. Through the use of papain-digested IgG_1 mAb, fractionated with both the 4′-TerPSEA and Protein A adsorbents, it was found that this IgG_1 mAb preferentially bound to the immobilised 4′-TerPSEA Sepharose FF™ resin through its F_c region.

Introduction

Monoclonal antibodies (mAbs)[1] are increasingly used for multiple therapeutic purposes, including the treatment of cancers and autoimmune diseases [1–4]. It is predicted that over the next decade monoclonal antibody developments will dominate the biopharmaceutical market [5], with sales of mAb therapies expected to reach $70 billion by 2015 [6,7]. Manufacture of these biopharmaceuticals involves a combination of upstream and down-stream processes, each requiring optimisation [8]. The downstream processes include a combination of capture, concentration, purification and formulation unit operations. Whilst there have been vast advances in recent years in mAb production, with large scale technologies developed for the culture of mammalian cells with very high expression yields, the recovery and purification of each specific mAb still presents important challenges [7].

Monoclonal antibody purification is a multistep process, often now involving application of platform procedures based on affinity chromatography using immobilised bacterial F_c receptors, such as Protein A from *Staphylococcus aureus* [9,10], Protein G from various *Streptococci* species, Protein L from *Peptostreptococcus magnus*, or even peptide or chemical mimetics [11–14], as the capture step followed by further purification and polishing steps involving, for example, ion exchange or hydrophobic interaction chromatography [7,8]. Protein A-based resins, the predominant mAb platform, are relatively expensive [15] and can account for 50% of the entire downstream processing costs [7]. In addition, the low pH elution conditions required to disrupt the mAb-Protein A complex can lead to degradation of both Protein A and the target mAb, and may not be compatible with subsequent downstream steps [16,17]. Formation of high molecular weight mAb aggregates, leaching of Protein A upon elution of the target mAb, incomplete removal of host cell proteins (HCP) and retention of viruses, lipids and nucleic acids are known to occur with Protein A adsorbents [18–21]. To minimise these constraints, a considerable effort has been expended by researchers in industry and academia over the past decade to optimise this procedure, with the consequence that Protein A affinity chromatography has emerged as the dominant platform technology for mAb purification. However, the search for alternative low molecular weight ligands has also gone on unabated in order to provide systems of higher productivity, improved economics and greater versatility. Moreover, not all IgGs from different species [18,22] bind to the Protein A based resins, e.g., rat IgGs bind only weakly, whilst some mouse IgGs and humanised IgGs do not bind at all to Protein A [18].

Efforts have thus been made in recent years to develop mixed mode chromatographic procedures for mAb purification [5,17].

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[1] *Abbreviations used:* 4′-TerPSEA, 4′-terpyridinylsulfanylethylamine; mAbs, monoclonal antibodies; HCP, host cell proteins; CHO, Chinese hamster ovary.

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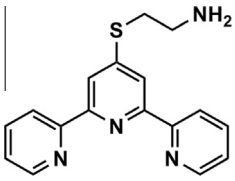

Fig. 1. Structure of the mixed mode ligand, 4′-terpyridinylsulfanylethylamine, 4′-TerPSEA.

Mixed mode chromatography exploits multiple physiochemical attributes of a ligand, through the interplay of electrostatic, dipole–dipole, hydrogen bonding and hydrophobic interactions [17]. The potential advantages of ligands that exhibit mixed mode binding attributes include scope for different selectivities, decreased costs, and the option for tandem use in multiple chromatography steps, with enhancement of protein purity [17,23]. In previous studies, we have described the synthesis of a family of pyridine-based derivatives and examined their potential as ligands for use in affinity and mixed mode chromatography for the purification of recombinant proteins [24,25]. Several of these compounds, when immobilised to suitable support materials, showed high binding affinities for mAbs, allowing their successfully capture and purification. Moreover, we have demonstrated that an additional member of this ligand family, namely 4′-TerPSEA (Fig. 1) can also be used for the purification of histidine-tagged recombinant proteins via immobilised metal ion affinity chromatographic procedures [26]. Thus, the selection of the specific 4′-terpyridinylsulfanylethylamine ligand employed in this investigation was based on several factors, including its relatively high binding affinity as assessed from screening studies with other mixed mode ligands [24,25], its responsiveness to changes in charge induction pH conditions, its facility for immobilisation via a terminal amino group without perturbation of the sulfanylethyl structure and potential to also act as a generic ligand useful in other modes of chromatographic interaction, such as immobilized metal ion affinity (when chelated with borderline metal ions) [26]. In this current investigation, several important parameters related to the mAb binding behaviour of 4′-TerPSEA-based resins when operated with mixed modality characteristics have been investigated from the perspective of capacity, selectivity and recyclability, with the chromatographic performance directly compared to a Protein A adsorbent using a humanised IgG_1 mAb.

Materials and methods

Synthesis and immobilization of 4′-TerPSEA

The ligand 4′-TerPSEA (Fig. 1) was prepared from the reaction of cysteamine hydrochloride with 4′-chloro- or 4′-iodo-2,2′:6′,2″-terpyridine with sodium hydride as the base as described previously [24,27]. Immobilisation of the ligand onto epoxy-activated Sepharose FF™ was based on previously described methods [24]. Briefly, Sepharose FF™ was activated using epichlorohydrin at 28 °C for 21 h. A 0.2 M solution of the ligand as the free base (20 mmol) was prepared by dissolving the ligand in 75% (v/v) aqueous MeOH (100 ml). Typically, the washed and suction-dried epoxy-activated Sepharose FF™ gel (100 g) was then added to this solution (100 ml) and the suspension gently mixed on a shaking platform at 28 °C for 21 h. The resulting ligand immobilised gel was collected by vacuum filtration, washed thoroughly with MeOH (8 × 100 ml), 50% (v/v) aqueous MeOH (100 ml), 25% (v/v) aqueous MeOH (100 ml) and water (5 × 100 ml) and stored in 20% (v/v) ethanol/H_2O (v/v) at 4 °C. The density of the ligand on the matrix was determined by elemental nitrogen analysis (Dairy Tech. Services, Melbourne, Australia) and found to be 271 μmol/g resin (dry weight).

Chromatographic purification of IgG_1 mAb using 4′-TerPSEA-Sepharose FF™

The 4′-TerPSEA resin was packed into Tricorn 5/50 columns (1 ml, GE Healthcare, Sydney Australia) with the chromatographic separations carried out with an ÄKTA purifier (GE Healthcare) at a flow rate of 1 ml/min. The crude IgG_1 mAb cell culture supernatant derived from Chinese hamster ovary (CHO) cell culture (kindly provided by Dr. Christine Bruun Schoidt, Novo Nordisk A/S) was diluted 5-fold with a higher strength buffer to achieve a final buffer condition that had the same ionic strength as Buffer A (25 mM Tris, 600 mM Na_3citrate, pH 8.0). This diluted cell culture supernatant (10 ml) was loaded onto the column and the column washed with Buffer A (15 ml). Protein elution was achieved by a step elution with Buffer B (25 mM MES, pH 5.0) (20 ml).

Chromatographic purification of IgG_1 mAb using MabSelect SuRe Protein A

HiTrap MabSelect SuRe Protein A columns (1 ml, GE Healthcare) were employed with the chromatography carried out using an ÄKTA purifier (GE Healthcare) at a flow rate of 1 ml/min. The same crude IgG_1 mAb cell culture supernatant was diluted 5-fold with a higher strength buffer to achieve a final buffer condition that was the same ionic strength as Buffer C (20 mM NaH_2PO_4, 150 mM NaCl, pH 7.2). This diluted cell culture supernatant (10 ml) was loaded onto the column which was then washed with Buffer A (15 ml). Protein elution was achieved by a gradient from 0% to 100% Buffer D (100 mM Na_3citrate, pH 3.3) (10 ml), followed by step elution with Buffer B (10 ml).

SDS–PAGE analysis of protein samples

Protein samples were diluted in 4× NuPAGE® loading buffer (Life Technologies, Sydney Australia) and protein bands separated by electrophoresis using the Hoefer miniVE vertical electrophoresis system (GE Healthcare, Sydney Australia) in 4–12% Bis-Tris NuPAGE® gradient gels (Life Technologies). Proteins were visualised by Coomassie blue staining.

Antibody loading capacity of Protein A and 4′-TerPSEA Sepharose FF™ columns

The IgG_1 mAb was firstly chromatographically purified with the Protein A adsorbent or the 4′-TerPSEA Sepharose FF™ as described above. The eluted fractions from several runs were pooled and dialysed to Buffer A for the subsequent experiments to be carried out with the 4′-TerPSEA Sepharose FF™ or to Buffer C for use with the Protein A resin. The concentration of the IgG_1 mAb sample was adjusted in each case to 2 mg/ml, and then continuously loaded onto the 4′-TerPSEA Sepharose FF™ or Protein A columns (1 ml) using an ÄKTA purifier (GE Healthcare) at a flow rate of 1 ml/min. until the frontal curve reached a plateau. For each resin, the volumes of the IgG_1 mAb solution required to achieve a tenth or half of the breakthrough plateau height was recorded and the amount of IgG_1 mAb contained in this volume calculated as the 10% or 50% breakthrough value for each respective column.

Reusability of the 4′-TerPSEA Sepharose FF™ columns

To test the reusability of the 4′-TerPSEA Sepharose FF™ columns, an ÄKTA explorer (GE HealthCare) was programmed for each column to run 25 cycles, each cycle according to the protocol described above. Clean-in-place (CIP) procedure was employed between each cycle. Thus, after elution of the mAb, the column was washed successively with 10 ml of each of the following