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Research paper

Affinity purification of IgG monoclonal antibodies using the D-PAM synthetic ligand: chromatographic comparison with protein A and thermodynamic investigation of the D-PAM/IgG interaction

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Abstract

This study investigates the applicability of D-PAM, the inverso form of the Protein A Mimetic synthetic peptide affinity ligand (PAM) obtained from the screening of a multimeric combinatorial peptide library, in monoclonal IgG isolation from ascitic fluids and cellular supernatants. D-PAM affinity columns, prepared by immobilizing the all-D peptide on the commercially available support Emphaze™, were able to capture monoclonal antibodies in a single chromatographic step, with a recovery yield and purity degree above 90% and full recovery of antibody activity.

D-PAM/Emphaze resin showed a host cell protein (HCP) and DNA reduction similar to protein A sorbent. Indeed, column capacity, determined by applying a large excess of purified antibodies to 1 mL of column bed volume, was always higher than 50 mg/mL.

D-PAM/IgG interaction was characterized by isothermal titration calorimetry (ITC) and an analysis of binding isotherms, obtained for titration of ST2146, ST1485 and 7H3 IgG monoclonal antibodies, suggested that two peptides bind simultaneously to the IgG molecule, with a K_A (equilibrium association constant) of 3.4, 6.2 and 3.4×10^4 M^{-1}, and a ΔH (change in enthalpy) of −1.3, −4.2 and −4.1 kcal mol^{-1}, respectively.
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Keywords: IgG; Monoclonal antibody; Affinity purification; Synthetic peptide ligand; Host cell proteins; DNA

1. Introduction

Monoclonal antibodies (MAb) represent the largest and most rapidly expanding category of biopharmaceuticals today and are useful for treatment of a wide array of indications including autoimmune diseases, infectious diseases, cardiovascular diseases, transplant rejection, and cancer (Huston and George, 2001; Groothuis and Nishida, 2002; Longo, 1996; Toi et al., 2004; De Santis et al., 2006). Many monoclonals are awaiting FDA approval and represent almost 30% of the biotechnology-derived drugs under development. Production of MAb by hybridoma technology or the use of transgenic animals can be easily scaled up, yet immunoglobulin purification from crude feedstock poses several problems. The main difficulties are due to the low antibody concentration in cell culture supernatants or milk of transgenic animals and the excessive amounts of contaminating proteins. Although IgA, IgE, IgM and

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IgY increasingly find application in the cure and/or diagnosis of important diseases, the majority of monoclonals under development for clinical application belong to the immunoglobulin G class. Purification of monoclonal antibodies of the G class for therapy is mainly based on the use of Protein A affinity ligand, immobilized on appropriate supports, as a first step in capturing and concentrating the immunoglobulin from diluted feedstock (Fuglistaller, 1989; Godfrey et al., 1993; Chou and Chen, 2001; Fahrner et al., 1999; McCue et al., 2003; Iyer et al., 2002; Aldington and Bonnerjea, 2007).

Protein A, which bind to the Fc portion of immunoglobulins and thus can be used to purify a majority of antibodies, is obtained from microorganisms or genetically modified bacteria through complex and expensive procedures. Such procedures require time-consuming analytical controls to check for the presence of contaminants, such as viruses, HCP, pyrogens or DNA fragments, which may affect the safety of the purified MAb for clinical purposes. The increasing number of monoclonals being developed for therapy and the role of the purification process in assuring the quality, consistency and safety of the products, prompted research activities to focus on the identification of novel synthetic ligands with improved properties (Huse et al., 2002; Lowe et al., 2001; Roque et al., 2004), in terms of both stability and regulatory points of view, since they can be sanitized and regenerated under very stringent conditions. Moreover, these synthetic molecules help to avoid the risk of contamination associated with natural ligands of human or animal origin (Bogdan et al., 2003).

To date, a number of synthetic derivatives have been proposed for immunoglobulin purification, such as peptides (Ehrlich and Bailon, 2001; Jacobs et al., 2003), amino acids (Bueno et al., 1995), thiols (Boschetti, 2001), dyes (Lowe and Pearson, 1984) and triazine-based ligands (Li et al., 1998). However, lack of selectivity for antibodies limits the widespread use of these ligands, which also require buffers not always compatible with retention of antibody activity. Recent investigations, based on the utilization of combinatorial technologies or computer modeling, provide novel compounds with very promising characteristics and the potential of becoming effective ligands for antibody purification, even on a large scale (Kabir, 2002).

We previously described a synthetic peptide, denoted PAM (Protein A Mimetic) or TG19318, and its inverso derivative, called D-PAM, where all amino acids are in D configuration, both able to specifically and selectively bind immunoglobulins of different classes and species, including IgG, IgE, IgA, IgM and IgY (Fassina et al., 1996; Palombo et al., 1998a,b,c; Verdoliva et al., 2000). PAM/Ig interaction was extensively investigated in affinity chromatography applications, and a number of peptide-derivatized matrices for antibody purification are now commercially available (Verdoliva et al., 2002; Michaelsen et al., 2003; Yan et al., 2002).

In this study, we extended the characterization of D-PAM, by investigating its applicability in monoclonal antibody purification, evaluating and comparing its ability to capture IgG directly from ascitic fluids and cellular supernatants with commercial protein A sorbents, and determining the optimal parameters to achieve high IgG recovery, functional activity and purity, also in terms of HCP and DNA reduction. In addition, to further characterize the interaction between D-PAM and immunoglobulins, isothermal titration calorimetry (ITC) was used to evaluate the thermodynamic parameters [stoichiometry of the interaction (N), equilibrium association constant (K_A), and change in enthalpy (ΔH)] related to the binding of D-PAM to ST2146 (IgG2b), ST1485 (IgG1) and 7H3 (IgG1) monoclonal antibodies.

2. Material and methods

2.1. Chemicals and biologicals

Anti-mouse IgG (Fc-specific) alkaline phosphatase, anti-mouse IgG alkaline phosphatase, anti-mouse IgG horseradish peroxidase (HRP), α-chymotrypsin, trypsin, papain and all chemicals were purchased from Sigma-Aldrich (Milan, Italy), unless otherwise stated. The 3 M Emphaze Biosupport Medium with Azalactone groups was purchased from PIERCE (Rockford, IL, USA). Prepacked recombinant protein A/Sepharose Fast Flow and Sepharose Fast Flow preactivated with *N*-hydroxysuccinimide (NHS) groups were purchased from Amersham Biosciences (Uppsala, Sweden). Total DNA assay and Threshold System were from Molecular Device Corporation (Sunnyvale, CA, USA). SP 2/0 Host Cell Protein (HCP) ELISA kit was from Cygnus Technologies (Southport, NC, USA). Tenascin and all monoclonal antibodies were from Tecnogen (Piana di MonteVerna, CE, Italy).

2.2. Monoclonal antibody production

Production of monoclonals was carried out through cultivation of corresponding hybridoma cells in protein-free (ST2146, ST2485) or fetal calf serum (FCS)-supplemented medium (7H3, ST1485 and 9B11), in a 2 L perfusion MD2 bioreactor (Sartorius BBI Systems,

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