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Research paper

Trace level analysis of leached Protein A in bioprocess samples without interference from the large excess of rhMAb IgG

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ABSTRACT

Resins containing immobilized Staphylococcal Protein A (PA) are widely used in the commercial purification of recombinant human monoclonal antibody (rhuMAb IgG) biotherapeutics. Therefore, a sensitive assay for leached PA is needed to ensure that PA is not present at unacceptable levels as an impurity in the final product. PA impurities are measured by an ELISA using chicken anti-PA antibodies. However, PA in the presence of IgG product forms a PA/IgG complex that interferes in the assay. In this report a multi-product PA ELISA is described, wherein the PA/IgG complex is dissociated by heating in the presence of detergents and chelators prior to the ELISA. The dissociation facilitates the accessibility of the anti-PA antibodies to bind to PA in the immunoassay. Heat is provided by a novel microwave technology which allows brief heating time and high sample throughput using a microtiter plate for sample heating. Thus, broadly applicable dissociation conditions, suitable for all 21 rhMab IgGs tested to date were identified. This approach streamlines the measurement of leached PA, allows higher sample testing throughput, facilitates application across multiple products, and facilitates assay automation. Data comparing in-process samples tested with both the former product-specific ELISA and this new multi-product assay are shown.

1. Introduction

Recombinant monoclonal antibodies are an important class of biopharmaceuticals, widely used to treat diseases such as cancer and autoimmune diseases. Commercially, recombinant monoclonal antibodies often are purified by affinity chromatography using resins immobilized with Protein A (PA) ligand (Jungbauer and Boschetti, 1994; McCue et al., 2003; Jungbauer and Hahn, 2004). PA affinity chromatography offers the benefits of high selectivity and high capacity, achieving high product purity in a single chromatography step. However, the PA ligand may leach from the affinity resin and co-elute with the monoclonal antibody product. PA may have immunogenic (Gomez et al., 2004) and mitogenic effects (Bensinger et al., 1984; Bertram et al., 1985; Kraft and Reid, 1985); therefore, immunoglobulin clinical products must be shown to be free of detectable trace PA impurities.

This paper compares two immunoassays used for trace PA quantification using the chicken anti-PA antibodies. Leached PA is quantified by an enzyme-linked immunosorbent assay (ELISA) which uses chicken IgY raised specifically against PA. Unlike IgG, the Fc portion of IgY does not bind to PA. The first assay is the original product-specific PA (PS-PA) assay, and the second is the newly developed multi-product PA (MP-PA) assay.

The samples being tested for trace levels of leached PA contain a vast excess of the IgG biotherapeutic. In the PS-PA assay, the samples are tested without pretreatment. Little, if any, PA is free in solution, but rather present in PA/IgG complexes. While PA binds to the product IgG primarily via Fc

Abbreviations: AD, Assay diluent; BSA, Bovine serum albumin; DMAD, Dissociation maintenance assay diluent; DTPA, Diethylenetriaminepentaacetic acid; ELISA, Enzyme-linked immunosorbent assay; IgG, Immunoglobulin G; IgY, Immunoglobulin Y; MP-PA, Multi-product Protein A assay; PA, Protein A; PCDB, Protein (or Protein A/IgG) complex dissociation buffer; PS-PA, Product-specific Protein A assay; rhMAb, recombinant humanized monoclonal antibody; SDS, Sodium dodecyl sulfate.

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region there are also binding sites in the Fab region (Sandor and Langone, 1982; Moks et al., 1986). As will be demonstrated, these IgG interactions with PA interfere with the binding of IgY anti-PA, thus interfering in the ELISA. Furthermore, highly homologous, but different, IgG therapeutics inhibit the ELISA to differing degrees, requiring that the assay standards and controls are diluted in the specific IgG product to a level equivalent to that in the samples. The requirement that the current PS-PA assay must control for unique product-specific inhibition effects limits assay throughput, and requires that the assay be optimized for each new rhuMab introduced into the product pipeline.

As such, a MP-PA method was developed, wherein the PA/IgG complex is dissociated prior to the ELISA (Fig. 1). This dissociation does not denature PA to the extent that recognition by the anti-PA IgY antibodies is compromised. This MP-PA ELISA is independent of the specific IgG in the sample and, therefore, may be applied across multiple rhuMab therapeutic products without the need for product-specific standards and controls. Also, the product independency of MP-PA ELISA requires minimum assay optimization for each new rhuMAb.

2. Materials and methods

2.1. Reagents and buffers

The following reagents were used in this study: ProSep vA (Millipore, Billerica, MA); MabSelect and MabSelect SuRe (GE Healthcare, Piscataway, NJ); Chicken anti-Staphylococcal PA (Cat. No. C5-B01, OEM Concepts, Saco, ME); Protein A/IgG Complex Dissociation Buffer (PCDB) — 8.5 mM Diethylenetriaminepentaacetic acid (DTPA)/1.5% BSA/0.1 M Sodium Phosphate Buffer/1% SDS pH7.2; Assay Diluent (AD) — 0.15 M Sodium Chloride/0.1 M Sodium Phosphate/0.1% fish gelatin/0.05% Polysorbate 20/0.05% Proclin 300 pH 7.2; Dissociation Maintenance Assay Diluent (DMAD) — 0.15 M Sodium Chloride/0.1 M Sodium Phosphate/0.1% fish gelatin/0.05% Polysorbate 20/0.05% Proclin 300/1% polyvinylpyrrolidone (PVP)/0.15% SDS pH 7.2. 96-well plate polymerase chain reaction (PCR) plate (Cat. No. 72.1978.202.96, Sarstedt, Germany).

2.2. Instruments

DISCOVERY, a temperature and pressure controlled microwave and MARS, a temperature controlled microwave (both from CEM Corporation, Matthews, NC); micro-plate reader SpectraMax M5^e (Molecular Devices, Sunnyvale, CA); Agilent 2100 Bioanalyzer and Protein 230 kit (Agilent Technologies, Inc. Santa Clara, CA).

2.3. Software

SoftMax Pro (Molecular Devices, Sunnyvale, CA), JMP statistics software, (SAS institute, Cary NC); Agilent 2100 Expert Software (Agilent Technologies, Inc. Santa Clara, CA).

2.4. Assay procedure

Samples were diluted in PCDB with a ratio of 1:5 in a 96-well PCR plate prior to heating in microwave under defined conditions, e.g. at 80 °C for 10 min. The plate was then centrifuged briefly (650 g, for 2 min) and 5 µL from the supernatant of each well was transferred into 95 µL DMAD in the wells of an ELISA plate that was precoated with anti-PA antibody at 4 µg/mL and blocked with AD. The plate was then incubated with shaking for 2 h at ambient temperature and then washed three times with PBS-T (PBS/0.05% Tween-20) before incubating with 100 µL HRP-anti-PA conjugate (70 ng/mL) for 1 h at ambient temperature. The plate was washed as before, and100 µL TMB substrate was added. Color development was

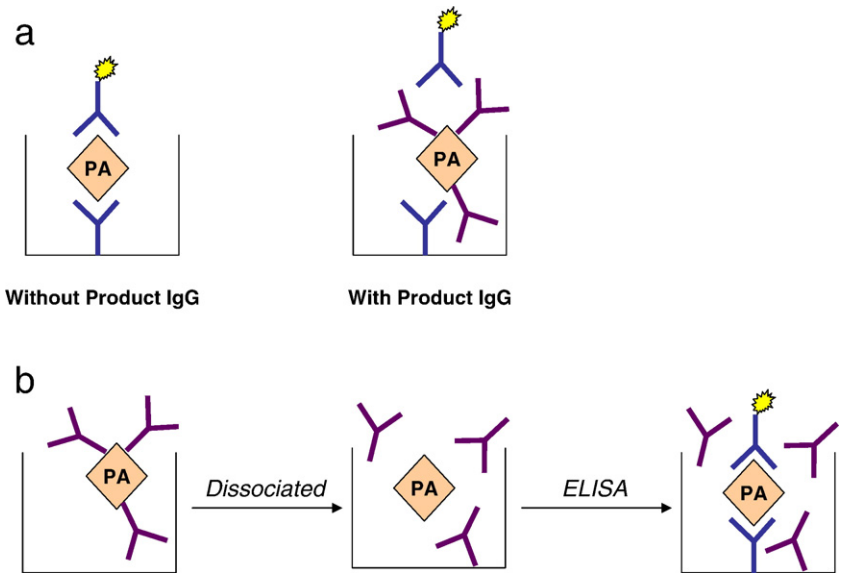

Fig. 1. Schematic drawing for the PA/IgG complex and its dissociation. a, PA forms a sandwich with its capture antibody (immobilized to the well of a microtiter plate) and detection antibody (blue color) in the leached PA ELISA; and in the presence of product rhMAbs (purple color), they form a PA/IgG complex. b, The PA/IgG complex is dissociated to allow full accessibility of PA for detection by the assay.