A METHOD FOR ANALYSING A SAMPLE OF IMMUNOGLOBULIN MOLECULES

Field of the Invention

The present invention relates to methods for analysing a sample of immunoglobulins, to related peptides, and to kits for carrying out such methods.

Background of the Invention

The characterisation of antibodies, such as structural characterisation and physiochemical analysis, is required by developers and producers of antibody based therapeutics. Mass spectrometry (MS) is one of the key analytical tools for characterizing therapeutic monoclonal antibodies (MAbs). Mass spectrometry in conjugation with HPLC is commonly used for studying the primary structure as well as post translation modifications (PTMs) and glycan structures of these large biomolecules. The large size of MAbs (150 kD) together with post translational modifications (PTMs) makes the analytical characterization of these biological therapeutics especially challenging.

For example, antibody-drug conjugates (ADCs) are an important class of therapeutic agent, which harness the selectivity of monoclonal antibodies (mAbs) to achieve targeted delivery of therapeutic agents. Critical to the clinical efficacy of an ADC are the target site-specificity and binding properties of the antibody, the *in vitro* and *in vivo* stability of the linker and the therapeutic agent, the potency of the therapeutic agent, and both the distribution and average number of therapeutic agents on the antibody. It is therefore important to understand the physiochemical properties of ADCs and develop analytical and bioanalytical techniques to assess and monitor ADCs during manufacture and subsequent storage.

Summary ~~of the Invention~~

Streptococcal erythrogenic toxin B (SpeB) is a cystein protease from *Streptococcus pyogenes*, shown to cleave IgG in the hinge region into two stable monomeric Fab fragments and one Fc fragment. In particular, SpeB has been reported to cleave human IgG between glycine residues 236 and 237. A second cystein protease from *Streptococcus pyogenes*, Immunoglobulin G-degrading enzyme of *S. pyogenes* (IdeS) has been reported to have an identical cleavage site for IgG as SpeB.

The inventors have carefully examined SpeB and IdeS activity on IgG and have made the surprising discovery that SpeB in fact cleaves IgG at a different site in the hinge region than IdeS. Developing this surprising discovery, the inventors realised that tandem cleavage of immunoglobulin with SpeB and IdeS allows the investigation of the hinge region of immunoglobulin molecules in more detail than was previously thought possible. This is important because the hinge region is a key site for post-translational modification and for conjugation of therapeutic agents in antibody-drug conjugates. Accordingly, in one aspect, there is ~~invention provides~~provided:

~~A~~ a method for analysing a sample of immunoglobulin molecules, comprising contacting the sample with a first polypeptide and a second polypeptide and analysing the resulting mixture, wherein the first polypeptide and the second polypeptide are cysteine protease enzymes which each cleave a different target site in the hinge region of human IgG;.

In another aspect, there is provided a~~A~~ peptide consisting of the amino acid sequence CPPCPAPELLG (SEQ ID NO: 3), or a variant of said sequence comprising one or two conservative modifications, preferably wherein said modifications occur only within positions 2 to 10 of said sequence; optionally wherein said peptide is conjugated to a therapeutic agent; ~~and~~.

In yet another aspect, there is provided a~~A~~ kit for use in a method of analysing a sample of immunoglobulin molecules, the kit comprising a first polypeptide and a second polypeptide as defined above.

Brief Description of the Figures

Figures 1A and 1B show results from SDS-PAGE following cleavage of the human monoclonal IgG1 antibody herceptin with IdeS (FabRICATOR) alone, SpeB (Fpn-1) and IdeS together, or SpeB alone.

Figure 2 shows a schematic overview of the different cleavage sites for SpeB (Fpn-1) and IdeS for human monoclonal IgG1 (Herceptin), as determined by mass spectrometry (LC/MS).

Figure 3 shows a schematic overview of cleavage of human IgG by SpeB (Fpn-1) giving rise to Fab and Fc fragments. The novel SpeB cleavage site at the hinge region is also indicated.

Figures 4A and 4B show a RP chromatogram with overlay of the hinge regions of peptides from avastin, herceptin and adcetris antibodies. In Figure 4B, a control synthetic hinge peptide has been added.