

Unique enzymes improve mass spectrometric analysis of IgG and makes it possible to use affinity purification of acid sensitive IgG molecules

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Introduction

The human pathogen *Streptococcus pyogenes* produces various surface proteins in order to avoid the host immune defence systems. Two of these proteins, EndoS (IgGZERO™) and IdeS (FabRICATOR™), have proven to be very useful as tools in antibody engineering. EndoS hydrolyzes the chitobiose core of the asparagine linked glycan on the heavy chain of IgG. IdeS is a cysteine protease that cleaves the IgG molecule at a unique site in the hinge region. We have also explored a mutated form of EndoS that binds selectively to the glycans of IgG.

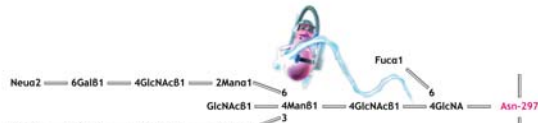


Fig 1. The endoglycosidase EndoS selectively cleaves off the glycan from IgG after the first N-acetylglucosamine attached to Asparagine 297.

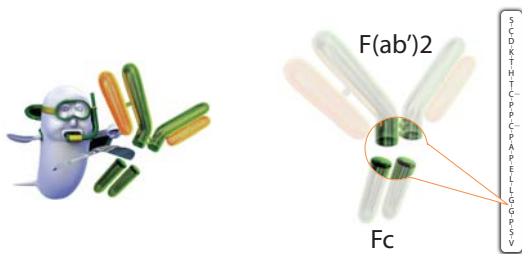


Fig 2. The proteolytic IdeS selectively cleaves IgG in the hinge region below the two thiol bridges thereby creating a F(ab)2 fragments and Fc-fragments.

Mass Spectrometry of IgG

When performing mass spectrometry of large proteins, such as IgG molecules, there is often a need to remove the glycans because of poor quality of the data otherwise obtained. Additionally, fragmentation of IgG can visualize individual parts of IgG and allow for studies in more detail. The obvious solution is then to remove the glycans attached to a Asp297 on the Fc part of IgG molecules. Among current methods, PNGase is used to accomplish enzymatic digestion of glycans to release them from the IgG. However, this is a process that requires several steps and long incubation times. We have evaluated a new endoglycosidase, EndoS (IgGZERO™) that selectively deglycosylates IgG molecules, for analysis in mass spectrometric applications. Due to its high specificity and activity, complete deglycosylation of IgG can be done in less than 30 minutes. Fragmentation is usually done enzymatically by using papain or pepsin which requires optimization and long incubation times. With IdeS, optimization is unnecessary and time is reduced significantly. Fragmentation is specific and completed in less than 30 minutes.

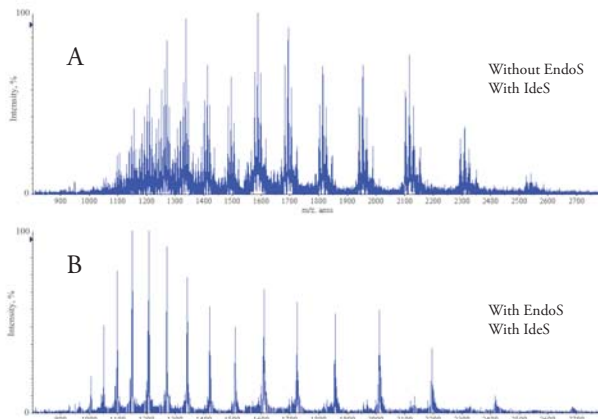


Fig 3. Mass spectrometry of Fc-fragments from human polyclonal IgG purified from plasma. (A) 1 mg of IgG was incubated with 1000 U of IdeS for 30 minutes and analyzed by mass spectrometry. (B) 1 mg of human IgG was incubated with 1000 Units of IdeS for 30 minutes followed by incubation with 1000 Units of EndoS for 45 minutes and finally analyzed by mass spectrometry. Utilization of RPC18 LC-ESI-MS generated a well-defined protein envelope with a deconvoluted mass of 24137 Da corresponding to the deglycosylated IgG1 heavy chain cleaved at position Gly236.

LS/MS of whole Herceptin® (Trastuzumab)

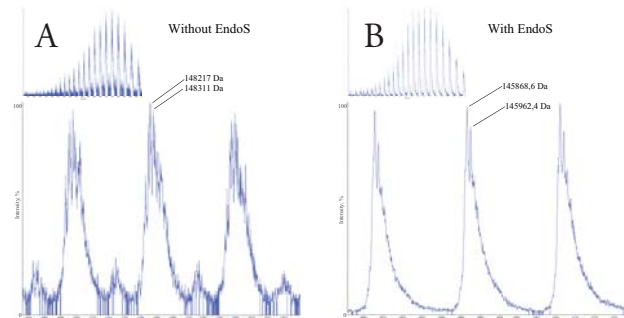


Fig 4. LC/MS of whole monoclonal IgG (Herceptin®, Trastuzumab) without (A) or (B) with prior treatment with EndoS for 30 minutes. Samples were analyzed directly after deglycosylation. With EndoS, a significantly increased quality of the MS-spectra was achieved, including clearly resolved masses of several protein variants.

LS/MS of F(ab)2 Herceptin® (Trastuzumab)

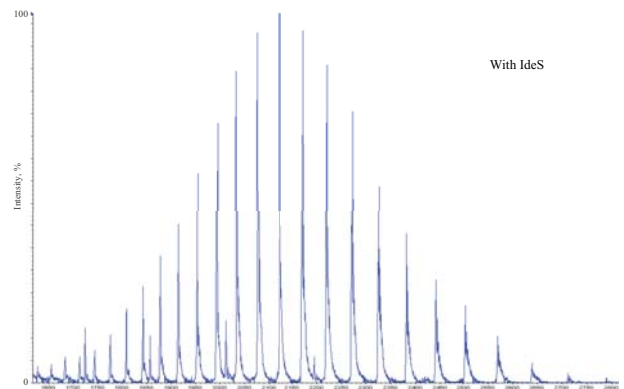


Fig 5. LS/MS of monoclonal IgG (Herceptin®, Trastuzumab) treated with IdeS for 30 minutes prior to LS/MS analysis. The molecular weight of the created fragments were determined to 97624 Da for the F(ab)2 fragments and 25232 Da for the glycosylated Fc-fragments.

Mutant EndoS binds to glycans of IgG

We have explored a mutated EndoS [rEndoS(E235Q)] that binds selectively to glycans of IgG. This property of rEndoS(E235Q) opens the possibility of using the mutated EndoS as an affinity ligand in purification of glycosylated IgG. This could be especially interesting for acid sensitive antibodies since elution conditions can be kept very mild.

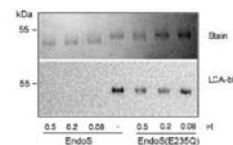


Fig 6. Dilutions of rEndoS or rEndoS(E235Q) incubated with 1 µg human IgG for 1 hour at 37°C. IgG-glycan hydrolysis by EndoS was analyzed by SDS-PAGE and LCA-blot.

Conclusions

The endoglycosidase EndoS releases IgG molecules from the attached glycans. This dramatically increases the quality of data obtained when IgG is analyzed by mass spectrometry. By combining EndoS with IdeS, individual fragments of IgG can be studied in more detail with higher accuracy and possibly facilitate amino acid sequencing for quality control of monoclonal antibodies. All three of these unique enzymes have potential to reduce time and cost of quality control and purification of monoclonal antibodies.