Zurich University
of Applied Sciences

Life Sciences and
Facility Management

Institute of Chemistry and
Biological Chemistry

Master Thesis 2011

Master of Science in Life Sciences
Chemistry for the Life Sciences

Top-down Strategy for the Investigation of Post-translational Modifications and Heterogeneity in IgGs

Master of Science Student
Roland Weibel

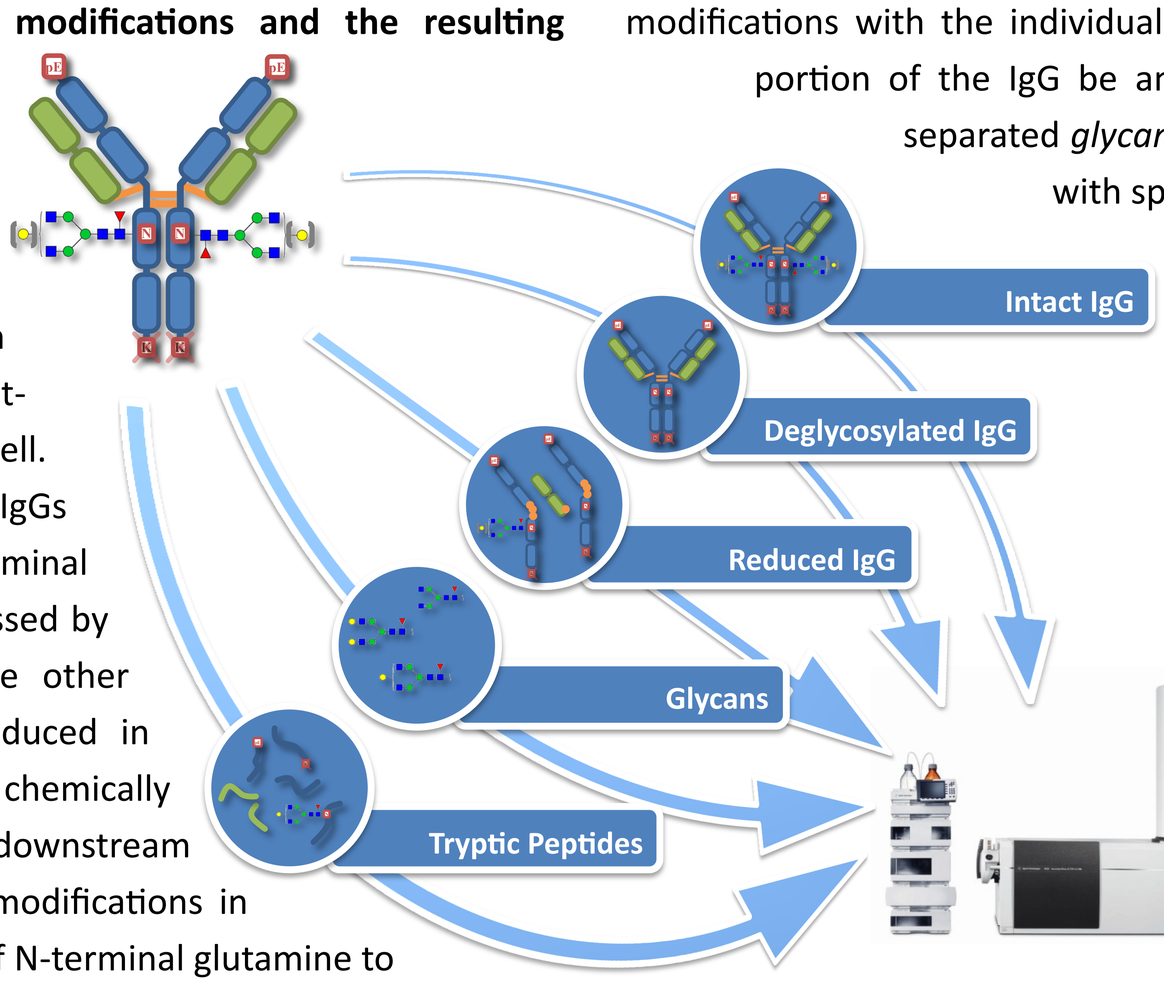
Lecturer
Prof. Dr. Christiane Zaborosch

Centre
Biochemistry

The physicochemical and biological properties of immunoglobulin G (IgG) are influenced by several modifications. These modifications can be analysed in detail by mass spectrometric methods. In a complex top-down analysis strategy, the modifications and the resulting heterogeneity in the monoclonal antibody XO01_γ1 were investigated.

Background

On the one hand modifications in IgGs already occur co- and post-translationally in the expressing cell. Thus, the glycosylation of most IgGs show heterogeneity and the C-terminal lysines can be enzymatically processed by a basic carboxypeptidase. On the other hand, monoclonal antibodies produced in mammalian cell lines can be chemically modified during upstream and downstream processing and storage. Example modifications in such cases include the cyclisation of N-terminal glutamine to pyroglutamate, the deamination of asparagine and the oxidation of methionine.



examined in more detail by *reducing* the IgG and determining the masses of the individual polypeptide chains before and after deglycosylation using RPLC-MS. This makes it possible to associate modifications with the individual subunits. Not only can the protein portion of the IgG be analysed with RPLC-MS, but also the separated *glycans*. The proteolytic splitting of the IgG with specific enzymes such as trypsin results in smaller peptides which can be very precisely analysed by mass spectrometry and assigned to the amino acid sequence of the IgG. In addition, the peptides can be fragmented by collision induced dissociation (CID) and the peptide sequence reconstructed from the mass of fragment ions. This usually enables exact assignment of each modification to an individual amino acid.

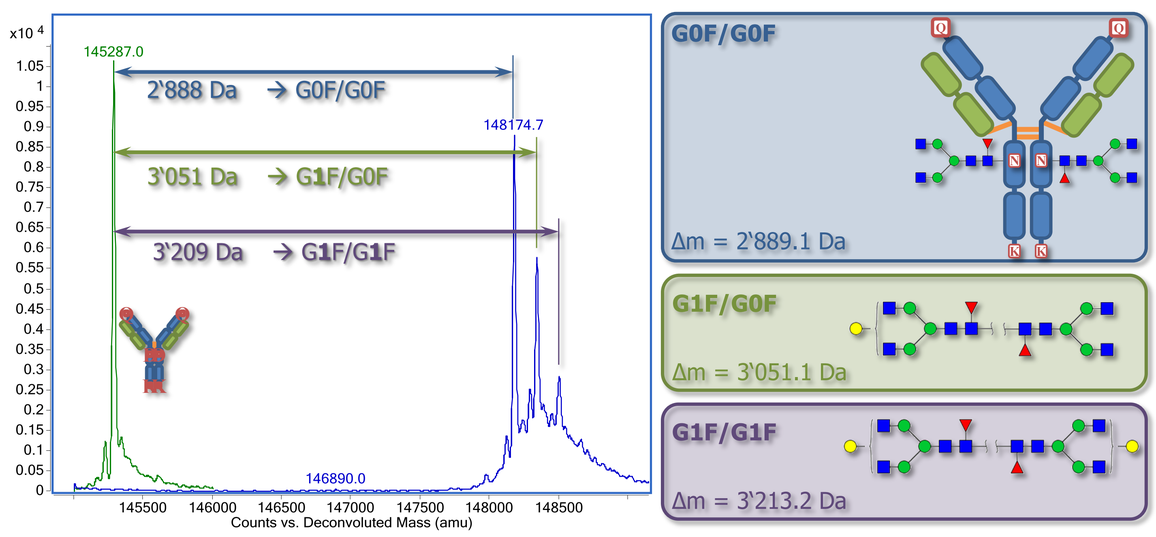


Figure 1: Deconvoluted mass spectrum of the intact and deglycosylated IgG XO01_γ1.

Modifications

The analysis strategy was exemplified using the monoclonal antibody XO01_γ1. G0F and G1F glycosylation were detected in the CH2 domain: this occurred in the combinations G0F/G0F, G1F/G0F and G1F/G1F on the two heavy chains (Fig. 1). The N-terminal glutamine of the heavy chains was completely cyclised to pyroglutamic acid. Furthermore, up to 97 % of C-terminal lysines were processed in these chains. Apart from these dominant modifications, deamination and oxidation were detected, but only in small amounts (Table 1).

Table 1: Detected modifications of XO01_γ1.

Seq. Loc.	Sequence	Modification	*Δm / Da*	%
HC(1-40)	**Q**VQLQESGPGLVKPSQTLSLTCSFSGF SLSTSGMGVGWIR	Cyclisation: Q(1) → pE	-17.0265	100 %
HC(252-258)	DTL**M**ISR	Oxidation: M(255) → M-Sulfoxide	+15.9949	1.6 %
HC(296-304)	EEQY**N**STYR	G0F N(300)	+1444.5339	75 %
HC(296-304)	EEQY**N**STYR	G1F N(300)	+1606.5867	25 %
HC(305-320)	VVSVLTVLHQDWL**N**GK	Deamination: N(318) → Suc	-17.0265	2.4 %
HC(305-320)	VVSVLTVLHQDWL**N**GK	Deamination: N(318) → D	+0.9840	1.3 %
HC(305-320)	VVSVLTVLHQDWL**N**GK	Deamination: N(318) → IsoD	+0.9840	4.0 %
HC(374-395)	GFYPSDIAVEWES**N**GQPE**N**NYK	Deamination: N(387/392) → Suc	-17.0265	0.3 %
HC(374-395)	GFYPSDIAVEWES**N**GQPE**N**NYK	Deamination: N(387/392) → D/IsoD	+0.9840	0.2 %
HC(420-442)	WQQGNVFSCSV**M**HEALHNHYTQK	Oxidation: M(431) → M-Sulfoxide	+15.9949	0.7 %
HC(443-449)	SLSLSPG**K**	Processing: K(450)	-128.0950	97 %
LC(93-103)	**M**LPWTFGQGTK	Oxidation: M(93) → M-Sulfoxide	+15.9949	1.9 %
LC(127-142)	SGTASVVCLL**N**NFYPR	Deamination: N(137) → D/IsoD	+0.9840	0.5 %

Top-down Strategy

Various methods of analysis were compiled into a top-down strategy for the study of modifications in IgGs. In the first step, the molecular mass of the *intact IgG* is determined by separating IgG from the sample matrix by reversed phase liquid chromatography (RPLC) and analysis in an ESI-Q-TOF-MS. To determine the molecular mass of the peptide part the IgG is *deglycosylated* and also examined using RPLC-MS. The subunits of the IgG which are connected via disulfide bridges are