6. SUMMARY

The sialylation of glycoconjugates is important for many cellular processes in cells and cell-cell contacts. Besides cell differentiation, inflammation, cell adhesion and pathogenesis, sialic acids influence the stability of glycoproteins. If these terminal monosaccharides are cleaved by sialidases glycoproteins bind to the asialoglycoprotein receptor and are degradated. The selective modification of monosaccharides should be tested for their influences on sialidase activities in comparison to native oligosaccharides.

The supplementation of CHO cells (murine) and HEK293, K-562 (both human) with 2-deoxy-d-galactose (2dGal) led to an exchange against subterminal galactose. In CHO cells the glycans of membrane proteins had 62% 2-deoxy-galactoses incorporated in a nearly complete sialidase A resistance. The human cell lines showed incorporation rates of 23-25% for 2dGal. In comparison to unmodified oligosaccharides a 1,6-fold increased resistance was determined in K-562. In the case of HEK293 glycans were more sensitive against sialidase A treatment. Generally 2dGal induced a reduction in glycosylation.

The also generated non-native sialic acids did not change the glycan profiles but the behaviour of these terminal monosaccharides. N-Acetylmannoseamines with modifications in the acetyl-group were used like native precursors of sialic acids and the resulting new neuraminic acids reached incorporation rates of 45-92%. The highest sialidase C resistance was detected for N-butanoyl- and N-pentanoylmannoseamine which also have shown the lowest incorporation rates. The new artificial monosaccharides led to an 2,5-fold higher sialidase resistance for CHO and 1,6-fold higher for HEK293 respectively. Both modifications, 2dGal and N-Acylmannoseamines, are good possibilities to modify the lifetime of glycoproteins.

Optimized masspectrometric methods and established fragmentation with iontrap techniques were used to characterize glycans without fluorescence labelling with HPLC separations. The Analysis of resulting data was standardised with programs which are freely available in the internet. For the calculation of molecular masses of glycans, peptides and glycoproteins a new tool GlycoProtMass was developed. This program can be used simultaneously to calculate masses of molecules and to save results with additional information.