

### VI Summary

Sialylation of glycoproteins and glycolipids on cell surfaces has an important role during development, differentiation and inflammation, as well as in the pathogenesis of diseases. Sialic acids are terminal components of oligosaccharides and involved in a variety of cellular interactions, such as cell-cell adhesion, cell migration and metastasis. They are also known to be involved in the formation of recognition determinants of pathogens and stability of glycoproteins. The *N*-acetylneuraminic acid is the biological precursor of all naturally occurring sialic acids. The first two steps, the epimerization of UDP-GlcNAc to ManNAc and the consecutive phosphorylation at C-6, are catalyzed by the bifunctional enzyme UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine kinase (GNE), the key enzyme of the sialic acid biosynthesis.

In this thesis two novel isoforms of human GNE, hGNE2 and hGNE3, have been identified. Opposed to hGNE1, hGNE2 possesses an extended and hGNE3 has a deleted N-terminus, respectively. GNE2 was also found in other species like apes, mouse, rat, chicken and fish, whereas GNE3 seems to be restricted to primates. Human and mouse isoforms displayed tissue specific expression pattern.

hGNE1, hGNE2, mGNE1 and mGNE2 were functionally expressed by the BAC-TO-BAC<sup>®</sup> baculovirus system. The 6xhis-tagged fusion proteins could be expressed as soluble active enzymes and purified by affinity chromatography in mg amounts. The hGNE3 protein was functionally expressed in *E.coli* BL21 cells, but its amount was very low. All recombinant proteins displayed ManNAc kinase activity. hGNE1, mGNE1 and mGNE2 formed UDP-GlcNAc 2-epimerase active tetramers. hGNE2 showed a 5-fold reduced UDP-GlcNAc 2-epimerase activity, which was due to the formation of dimers. hGNE3 displayed no epimerase activity at all.

In the last part of this thesis the proteins VCP and Oxr1 were analyzed for potential interactions with the GNE protein. The results implicated that neither VCP nor Oxr1 are interaction partners of GNE.