7 Summary

Cloning and expression of enzymes involved in the salvage pathway of L-fucose

L-fucose is an important monosaccharide moiety of various complex N- and O-linked glycans and glycolipids produced by mammalian cells. Fucose frequently exists as a terminal modification of oligosaccharide chains and plays an essential role in several biological processes. The biosynthesis of L-fucose takes place in the cytosol of mammalian cells. The *de novo* pathway uses GDP-mannose as a starting point. A multienzyme complex converts GDP-mannose to GDP-fucose. 10% of GDP-fucose derives from the salvage pathway, which reutilizes fucose from nutritional sources and from the degradation of fucosylated glycans. Two enzymes are involved in this pathway L-fucose kinase and GDP-fucose pyrophosphorylase.

In the present study two different systems for the expression of recombinant enzymes involved in the salvage pathway of L-fucose were used. Furthermore, the human fucokinase and their C-terminally located kinase domain itself were expressed as GST fusion proteins in *E. coli*. It was not possible to overexpress the complete GST-tagged fucokinase as a soluble protein, beceause all of the recombinant protein was found in inclusion bodies. The recombinantly expressed GST fucokinase kinase domain also formed inclusion bodies. However, a significant portion of the protein was soluble and was purified by affinity chromatography almost up to homogeneity. Additionally, inclusion bodies of the expressed murine fucokinase as a GST-tagged protein could be solubilized under denaturating conditions. The second expression system used was the baculovirus expression system, which is appropriate to the heterologous expression of mammalian proteins in insect cells. Nevertheless, the expression of fucokinase as a soluble protein was not successful. It is conceivable that the expressed protein here had a toxic effect on the insect cells. In contrast, the expression of GDP-fucose pyrophosphorylase was successful in insect cells. The soluble fraction of the cells contained up to 20% of recombinant protein.

In the second part of this study antiserum against the human fucokinase was generated. At the beginning of these experiments no recombinant proteins were available, and the immunization of rabbits with chemically synthesized peptides was necessary. These peptides consist of sequences of the primary structure of the fucokinase. Altogether six rabbits were immunized, and all attained antisera exhibited a positive reaction in the dot-blot analyses towards the selected peptide. Additionally, one of the two peptides antisera could identify rat fucokinase in the western blot analysis.

The results presented here offer the basis for further biochemical and immunohistological investigations, which could decode in more detail the role of the salvage pathway of fucose.