expressed by replacing the cytoplasmic and transmembrane domains with the signal sequence of yeast invertase. Activities were measurable in the 100.000 g supernatant of ABYS transformants, whereas activity of membrane forms was found predominantly in the pellet. Further work is aimed at scaling-up synthesis of the production of recombinant glycosyltransferases.

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S6.6

Comparison of the Posttranslational Modifications of Human Proteins Secreted from Different Baculovirus-Infected Insect Cell Lines

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Five different lepidopteran cell lines have been studied with respect to their capability of proteolytic processing, posttranslational modification with *N*- and *O*-linked carbohydrates and secretion of genetically engineered human model proteins expressed after infection with recombinant baculoviruses. *Spodoptera frugiperda* cell lines (Sf21 and Sf9) and cell lines obtained from *Trichoplusia ni, Estigmene agrea* and *Bombyx mori* were grown under standard laboratory techniques in roller bottles or in stirred tank perfusion reactors. Accumulation and quality of products were monitored in culture supernatants after different times post-infection of cells using bioassays, western blot analysis as well as (after purification of proteins) by amino acid sequencing and carbohydrate structural analysis.

Apart from different *N*-terminal truncations and intrachain proteolytic cleavage, incomplete leader peptide cleavage in the secreted products was observed. *N*-glycosylation analysis revealed a host cell-specific carbohydrate pattern of mainly the oligomannosidic type of glycan structure (Man2 – Man5) with a varying degree of proximal fucose. These structures are commonly not found in glycoproteins from mammalian origin. A varying degree in *O*-glycosylation with GalNAc or Galβ1-3GalNAc was observed in each cell line.

S6.7

Characterization of a Phosphorylated Oligosaccharide from Erythropoietin Expressed in Recombinant BHK Cells

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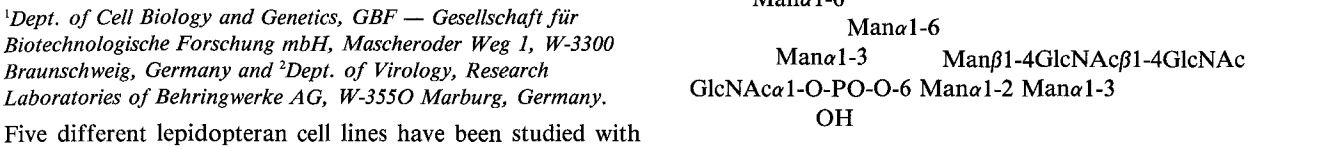
Erythropoietin (EPO), a glycoprotein with three *N*- (Asn24, 38, 83) and one *O*-glycosylation site (Ser126), is the principal regulator of hematopoiesis. Recombinant EPO from heterologuous mammalian cell lines is in clinical use for the treatment of chronic anemia due to renal failure.

During high pH anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) of the enzymatically released *N*-glycans from several batches of the recombinant protein produced in BHK cells a sialidase resistant oligosaccharide eluting in the region of the monocharged *N*-glycans was detected. Glycosylation site specific analysis showed it to be present mainly at Asn24. Large scale isolation of this structure was performed by enzymatic (PNGase F) release of the total *N*-glycans, separation of the oligosaccharides according to charge on MonoQ and further separation of the monocharged MonoQ fraction by HPLC on an aminobonded phase. The following high mannose type oligosaccharide incorporating a phosphodiester bridged additional GlcNAc residue was elucidated using methylation analysis, FAB-MS and 1D and 2D 600 MHz NMR experiments.



The possible implications of this structure for the metabolic fate of the glycoprotein will be discussed.

S6.8

Oligosaccharide Processing of Human Tamm-Horsfall Glycoprotein Permanently Expressed in HeLa Cells

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Human Tamm-Horsfall glycoprotein (T-H) is produced by renal cells of ascending limb of Henle's loop and is largely excreted in urine. It has been demonstrated that T-H is a glycophosphatidylinositol-anchored protein (Rindler *et al.*, *J. Biol. Chem.* 265, 20784-20789, 1990). The carbohydrate content of T-H is close to 30%. The oligosaccharide processing and maturation rate of recombinant T-H permanently expressed in HeLa cells were studied. Pulse chase experiments with [^{35}S]methionine of transfected HeLa cells showed a very slow conversion from precursor to mature T-H. The precursor disappears with a half time higher than 6 h. Glycans carried by precursor T-H labeled with [^{3}H]mannose (1 h pulse plus 2 h chase) have all the high-mannose structure and $Man_8GlcNAc_2$ is well represented, suggesting that a significant amount of precursor was not routed to the Golgi apparatus. Since T-H has a very high number of disulfide bridges (more than 50 cysteine residues/mol) one may infer that the low rate of export out of ER depends on a relative long time required for the formation of a correct set of disulfide bonds. Mature T-H isolated from HeLa cells after 20 h of labeling plus 3 h chase with [^{3}H]mannose retains one *N*-linked chain with the high-mannose structure similarly to urinary T-H. This result indicates that the occurrence of one unprocessed high-mannose chain in mature T-H is host-cell independent and very likely related to T-H primary structure. However in recombinant T-H Man_{7-8}-$GlcNAc_2$ chains predominate, whereas in the urinary T-H $Man_6GlcNAc_2$ is the