S5.21
Proteoglycans in Human Cartilage, Blood and Synovial Fluid

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Proteoglycans were prepared from human synovial fluid, blood and articular cartilage using a new method based on precipitation with Alcian Blue. Proteoglycans in a solution of 4 M guanidine-HCl can be specifically precipitated with the cationic dye Alcian Blue at low pH and in the presence of detergent. Neither proteins or unsulphated glycosaminoglycans are precipitated. The proteoglycan-Alcian Blue complexes are dissociated in a mixture of guanidine-HCl and propanol. The proteoglycans are precipitated by increasing the propanol concentration and the dye is used for quantitation and the proteoglycans can be separated by electrophoresis in gels of pure agarose, using a discontinuous buffer system that allows stacking of the sample. Large proteoglycans from articular cartilage were separated into three populations by electrophoresis. The synovial fluid contained three large size proteoglycan fragments but no small fragments or free glycosaminoglycans were found. In blood plasma only very small proteoglycans were detected. Electron microscopy of the proteoglycans from synovial fluid revealed fragments from both the *C*-terminal and *N*-terminal domains of the large proteoglycans. SDS-PAGE patterns of enzymatically deglycosylated proteoglycans extracted from the cartilage and from synovial fluid were compared. In both cases two major core protein sizes were found with several intermediate sizes, but the synovial proteoglycan fragments were smaller than the cartilage molecules.

S.6 RECOMBINANT GLYCOPROTEINS

S6.1
Recombinant Glycoproteins: Pitfalls and Strategy

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Glycoconjugates, which result from the covalent linkage of a sugar moiety called glycan with proteins (glycoproteins) or lipids (glycolipids), have acquired a great importance in the 20 past years thanks to a series of discoveries demonstrating the role played by the carbohydrate moiety. In fact, we know now that glycans 1) influence the conformation of peptide chain; 2) protect the protein against proteolytic attack; 3) diminish the immunogenicity of proteins and hormones; 4) are receptor sites for viruses, proteins and hormones; 5) regulate the catabolism of circulating proteins and determine the lifetime of proteins and cells; 6) control the social life of cell by intervening in intracellular adhesion and recognition and in cell-contact inhibition. In this connection, the profound modifications of the glycan structures observed in cancerous cell membranes could be a factor of cancer induction and metastatic diffusion. Thus, the knowledge of the role of glycans raises a formidable problem in the field of genetic engineering of human glycoproteins. In fact, prokariotic cells express only the protein part of a glycoprotein, being devoid of the convenient glycosyltransferase systems. So, the only solution consists to insert the cloned DNA in the genome of eukaryotic cells so that they express a copy of the glycoprotein which is in conformity with the native one. However the choice of the expression cell as well as the culture conditions deeply influence the glycan biosynthesis. This leads in many cases, to a non-optimal glycosylation of recombinant proteins of which the consequences are dramatic: increase of hydrophobicity, decrease or inhibition of the secretion level, decrease of the stability towards heat or proteases, shortening of the biological half-life by increase of the clearance, decrease of the affinity for specific receptors and increase of the antigenicity.

On the basis of the knowledge of the primary and tridimensional structure of glycans, their metabolism and molecular biology will be envisaged. The demonstration of the complexity of the biosynthetic pathway of glycans will reveal the difficulties and pitfalls encountered in the development of the "glyco-biotechnology" and in the production of recombinant glycoproteins.

S6.2
Development of a Recombinant Form of Ceredase® (Glucocerebrosidase) for the Treatment of Gaucher's Disease

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Ceredase® (macrophage-targeted glucocerebrosidase from human placenta) is a highly effective therapeutic for the treatment of Gaucher's disease. To increase the availability of this product a recombinant form of the enzyme is under development. Human glucocerebrosidase (GCR) was overexpressed in Chinese hamster ovary cells transfected with cDNA encoding GCR. The oligosaccharide chains of the purified recombinant enzyme were remodeled by sequential digestion with neuraminidase, β-galactosidase and β-hexosaminidase to produce mannose-terminated rGCR. The binding of the remodeled form of rGCR to the mannose receptors of macrophages was found to be saturable, inhibited by yeast mannan, and of high affinity (K_d = 3 nM). In addition, *in vivo* biodistribution studies in mice demonstrated that approximately 14% of the remodeled rGCR targets to Kupffer cells, an 8-fold improvement when compared with native placental GCR. A GlcNAc transferase I-negative (Lec1) mutant cell line expressing rGCR was isolated by treatment of the transfected cell line with wheat germ agglutinin and lentil lectin. The binding of purified rGCR from the Lecl mutant cell line to the mannose receptor of isolated rat peritoneal macrophages was comparable to that of the remodeled placental rGCR.

S6.3
Expression of Soluble Recombinant α1,3-Galactosyltransferase in Insect Cells