R. J. Harris[1], H. van Halbeek[2], J. Glushka[2], L. J. Basa[1], V. T. Ling[1], K. J. Smith[3] and *M. W. Spellman*[1]
[1]*Department of Medicinal and Analytical Chemistry, Genentech, Inc., 460 Pt. San Bruno Blvd., So. San Francisco, CA, USA.*; [2]*Complex Carbohydrate Research Center and Department of Biochemistry, The University of Georgia, Athens, GA, USA.; and* [3]*Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM, USA.*

O-linked fucose has been found attached to Thr/Ser residues within the sequence -Cys-X-X-Gly-Gly-Thr/Ser-Cys- in the *N*-terminal EGF modules of several coagulation/fibrinolytic proteins. Carbohydrate composition and mass spectrometric analyses of tryptic and thermolytic peptides containing the corresponding site (Ser-61) in the first EGF module of human factor IX indicated the presence of a tetrasaccharide containing one residue each of sialic acid, galactose, *N*-acetylglucosamine and fucose. Fragments generated during mass spectrometric analysis indicated that fucose was the attachment sugar residue. The complete structure of the tetrasaccharide was obtained by methylation analysis and 2D 1-H TOCSY and ROESY experiments to be NeuAcα-(2→6)Galβ(1→4)GlcNAcβ(1→3)Fucα1→*O*-Ser-61. The novel tetrasaccharide described here is found within a 12 amino-acid linear sequence of factor IX that contains two other posttranslational modifications (β-hydroxyaspartate and Xyl-Xyl-Glc glycans) found uniquely in EGF modules.

S9.11

Mass-Spectrometric Analysis of the Glycosylation of the Enzyme Lecithin-Cholesterol Acyl Transferase

A. J. Reason, H. R. Morris and A. Dell
Wolfson Laboratories, Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.

Lecithin-Cholesterol Acyl Transferase (L-CAT) is the enzyme responsible for producing cholesteryl esters in human plasma. The enzyme has been assigned to a key role in lipoprotein metabolism, however, little information concerning its function and mechanisms of action has been gathered. The cDNA derived amino acid sequence of the human enzyme has four potential glycosylation sites at asparagine residues 20, 84, 272 and 384, but little is known about the degree and type of glycosylation of this enzyme. Due to the establishment of a BHK cell line that secretes human recombinant L-CAT, (Karmin, O., Hill, J. S., Wang, X. B. and Pritchard, P. H., *J. Lipid Res.*, 34, 81–88) we now for the first time have sufficient amounts of purified enzyme available for detailed structural characterisation studies, and we have designed experiments to unequivocally determine the glycoforms of L-CAT. Initially the recombinant form of the enzyme was subjected, after reduction and carboxymethylation, to digestion with trypsin, and potential *N*-glycans were removed using PNGase F and permethylated. FAB-MS analysis of the released *N*-glycan population indicated the presence of fucosylated and non-fucosylated bi- and tri-antennary structures. These initial analyses were followed by detailed FAB-MS and ES-MS studies on individual *N*-linked glycopeptides and FAB-MS screening for *O*-glycosylation. Similar strategies are being used to determine whether the recombinant material expresses the same *N*-linked glycosylation pattern as naturally occurring plasma L-CAT.

This work was carried out in collaboration with Professor A. G. Lacko and colleagues (Texas College of Osteopathic Medicine) and Dr. P. H. Pritchard and colleagues (Department of Pathology, University of British Columbia).

We would like to thank the MRC, SERC and Wellcome Trust for financial support.

S9.12

The Role of Plasma Desorption, Electrospray and Matrix-Assisted Laser Desorption Mass Spectrometry in the Characterization of the Glycan Chains and Linkage Regions of Surface-Layer Glycoproteins

G. Allmaier[1], E. R. Schmid[1], B. Stahl[2], M. Karas[2], J. Schuster-Kolbe[3], U. B. Sleytr[3] and P. Messner[3]
[1]*Institute for Analytical Chemistry, University of Vienna, A-1090 Vienna, Austria;* [2]*Institute for Medical Physics and Biophysics, University of Münster, D-4400 Münster, Germany; and* [3]*Center for Ultrastructure Research and Ludwig-Boltzmann Institute for Molecular Nanotechnology, University of Agriculture, A-1180 Vienna, Austria*

Crystalline bacterial surface layers (S-layers), composed of protein or glycoprotein species, form the outermost cell envelope component of many prokaryotic and eukaryotic cells. In our continuous systematic survey on eubacterial S-layer glycoproteins, the molecular weight determination and structural elucidation of the glycan structures and the carbohydrate-protein linkage regions play a crucial role. Currently we are studying purified S-layer glycoproteins obtained after extensive pronase digestion from various strains of *Clostridium thermohydrosulfuricum*. In most cases these compounds consist of 1 to 3 amino acids *O*-linked to linear glycan chains with up to 100 monosaccharide units.

The exact molecular weight is the first requirement in the structural analysis of these underivatized substances and we evaluated plasma desorption (PD), electrospray (ES) and matrix-assisted laser desorption (MALD) mass spectrometry for this purpose. PD and ES turned out to be useful up to 3 kDa. MALD for the first time permitted us to determine the molecular weight for a glycan chain (consisting of 70 sugar units bound to a single amino acid) with an accuracy of ±0.05%. Therefore it is the method of choice. The MALD technique has the advantage of unsurpassed sensitivity, requiring only pmol amounts of material and is the only desorption/ionization able to generate sodiated molecular ions in the required mass range. Apart the molecular weight information, structural details as the protein-carbohydrate linkage and the glycan sequence are of great interest. PD and ES mass spectrometry can supply these data at least to some extent, based on fragment ions observed with both techniques. In case of PD we obtained a complex fragmentation pattern formed by a high energy collision process. By increasing the skimmer voltage in the ES source a simple, straightforward interpretable fragmentation pattern was generated similar to a low energy collision induced dissociation spectrum.

The role of these mass spectrometric techniques for S-layer glycoprotein analysis will be discussed in context of data obtained by 600 MHz nuclear magnetic resonance spectroscopy and GC/MS analysis.

This work was supported by grants from the Jubiläumsfond der Österreichischen Nationalbank: 4044 to G. A. and 4332 to P. M. Further financial support was granted by the Austrian Fonds zur Förderung der Wissenschaftlichen Forschung (P8922 to P. M.).