exclusion chromatography on a concanavalin A column. In further work, we used a crude bacterial enzyme preparation to produce large quantities of the deglycosylated form of the protein. The deglycosylated avidin was assumed to be totally devoid of sugar, based on chemical analysis and on the lack of interaction with lectins (i.e., concanavalin A and wheat germ agglutinin).

In the past, it has been difficult to obtain well-diffracting crystals of avidin for X-ray studies of its structure, perhaps due to the heterogeneous nature of the oligosaccharide moieties. The availability of a deglycosylated form of avidin has thus provided us with appropriate material for such studies. Indeed, we were able to obtain crystals of the deglycosylated protein which led to the elucidation of the three-dimensional structure of avidin and the avidin-biotin complex [3]. One of the surprises in this work was the discovery (on the basis of difference Fourier synthesis) of a residual *N*-acetyl glucosamine moiety in the deglycosylated avidin monomer.

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S9.5

SERS Spectroscopy — Physical Probing of Cell Surface Sialosides

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SERS (surface-enhanced Raman scattering) is high sensitive spectroscopic method to study peripheral layer of macromolecules. This work deals with the application of SERS method to study the number of sialosides including glycosides, natural sialoglycoproteins and synthetic pseudopolysaccharides in view of the fact that peripheral fragments of glycoconjugates are sialic acid residues.

Methyl and benzyl glycosides of Neu5Ac were characterised by specific SERS spectra (differed from other biomolecules) and unusually high intensity of signals compared with that of any uncoloured substance studied by SERS before. The loss of signal was observed after 8,9-acetonation of the sialoside indicating the C9-C7 glycerol moiety to contribute mostly to the SERS signal. A dependence of intensity of SERS spectra from Neu5Ac content of synthetic polymers was observed.

All asialoglycoproteins gave almost identical spectra with low signal intensity. SERS spectra of sialoglycoproteins depend on the number of sialylated chains, their type and antennary and bond type with penultimate galactose residue (2-3 or 2-6). Proteins having identical peptide core but differing in glycosylation gave spectra having pronounced differences as well as common features. Characteristic signal of neuraminic acid was obtained on one live myeloma cell, after neuraminidase treatment this signal disappeared.

S9.6

Site-Specific Glycosylation of the Influenza Virus Haemagglutinin

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Three variants of influenza A (H1N1) virus, strain WSN, have been previously identified, which when grown in MDBK cells, vary in their ability to bind to host cell receptors [1]. These variants are identical except for single amino acid differences in the haemagglutinin (HA) at residues 129 (N vs. D) and 184 (H vs. N) of HA1 [2]. One of these variants has N at both of these positions, creating a potential glycosylation site at 129, and has reduced receptor binding activity. The other two have N at either 129 or 184 and have high binding activity. When these three variants are grown in CEF instead of MDBK cells they are highly similar to each other in receptor binding properties.

We are using site-specific structural analysis of the HA oligosaccharides to investigate this host-mediated effect on receptor binding. We find that all of the potential glycosylation sites in the high binding variants (at residues 20, 65, 129 and 271 of HA1) are used by MDBK cells and that their glycosylation is site-specific. Laser desorption mass spectrometry of isolated tryptic glycopeptides and analysis of the oligosaccharides released by hydrazinolysis from the glycopeptides indicate that, of the sites common to the variants, 20 and 271 have very large complex oligosaccharides, whereas site 65 has predominantly oligomannose structures. Site 129 in the one case studied so far also has complex oligosaccharides, although they are much smaller than those at sites 20 and 271. The data suggest that receptor binding is reduced by the combined effect of MDBK-synthesised oligosaccharides at residue 129 on the surface of the HA and an H to N amino acid substitution at residue 184 adjacent to the receptor binding pocket.

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S9.7

Conformational Analysis of Antifreeze Glycoprotein

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At the first the spatial structure of the glycoprotein, explaining observed physical fact – abnormal depressing of freezing temperature – is predicted by theoretical conformational analysis.

O-glycosylated antifreeze glycoprotein (AFGP) of regular primary structure, the segment of which is pictured in an active, C_3-conformation, is founded in several species of fishes in northern seas, where notwithstanding extreme living conditions they survive. In the active conformation of left helix with 3-fold axis the disaccharide units Galβ1-3GalNAc in the side chains of the each third residue Thr are the factors not only for stabilization of the structure, owing to effective