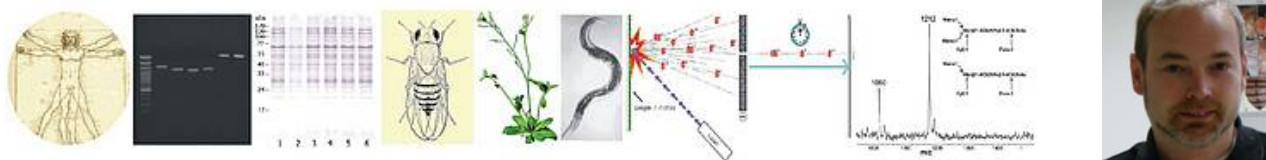


NOVARTIS-PREIS FÜR CHEMIE 2007

IAIN B. H. WILSON



Zusammenfassung:

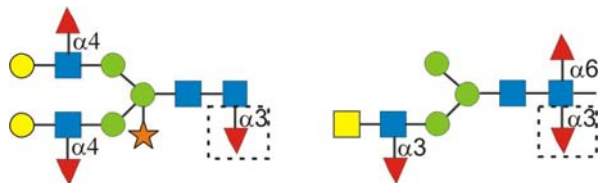
Der Novartispreis 2007 für Chemie ging an Iain Wilson von der Abteilung für Biochemie an der Universität für Bodenkultur Wien. Er studierte in Edinburgh und Oxford und war danach als Post-Doc an der BOKU und in Dundee beschäftigt. Anschließend kehrte er als Universitätsassistent an die BOKU zurück, wo er sich 2001 in Biochemie habilitierte; speziell die Erkennung von core- α 1,3-gebundener Fucose durch Antikörper und deren Rolle in Allergien wie auch die Parasitologie standen bereits da im Zentrum seines Interesses. In den vergangenen Jahren hat er eine Forschungsgruppe mit zurzeit zwei Post-Docs, zwei Doktoranden und einer Technikerin aufgebaut.

Sein Hauptaugenmerk liegt auf der Erforschung der Modifikation von Proteinen mit Zuckern, die speziell in Pflanzen, Fliegen, Würmern und Amöben interessante Variationen aufweisen. Das Interesse gilt vor allem dem Zusammenhang zwischen Fucose und Allergie, den Zuckerketten in Nematoden, die die Empfindlichkeit des Immunsystems senken, der Glykosylierung in Fliegen-Neuronen und der Veränderung von N-Glykanen während der Entwicklung von Schleimpilzen. Ausgehend von dieser Grundlagenforschung möchte seine Gruppe die Zuckerstrukturen von Parasiten nachbauen, um deren Effekte auf das Immunsystem studieren zu können. Ein weiterer Forschungsschwerpunkt ist das Re-Engineering von Insektenzellen, damit diese pharmakologisch relevante, jedoch nicht immunogene Glykoproteine produzieren.

The research details in English:

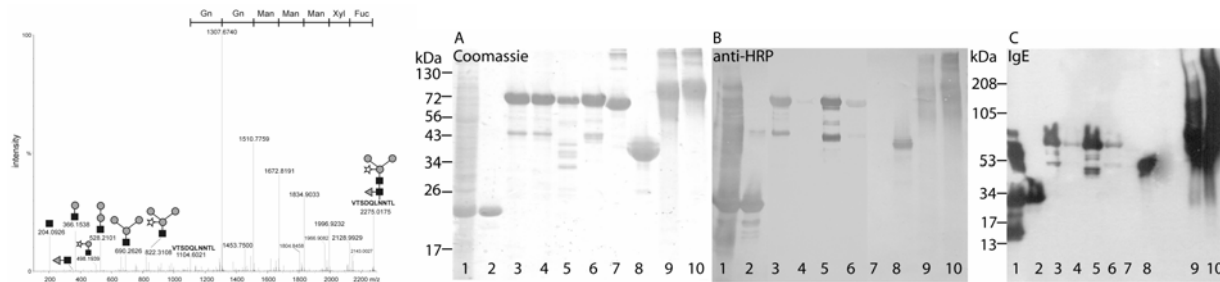
Glycobiology of invertebrates and plants with a focus on immunogenic glycans

Most of my research career has been related to the biomedical and immunological aspects of glycosylation. This class of post-translational modification of proteins with sugar chains is involved in many cell-cell and host-pathogen interactions. For the past decade or so, my major research focus to date has been the glycans and glycosyltransferases of invertebrates and plants with a particular interest in those structures which are immunogenic in mammals. This type of work has a long history at the BOKU, having been initiated by Prof. Leopold März and his group; a focus being on the core α 1,3-fucose modification of N-linked (asparagine-linked) sugar chains.



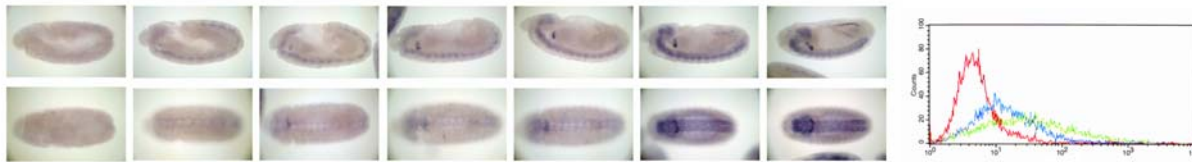
Pictorial representations of example complex N-linked glycans from plants (left) and insects (right); the fucose residues are shown in red and that the core α 1,3-fucose is boxed).

In a series of studies on a number of vegetables, fruits and pollens, we could observe that core α 1,3-fucose is present on N-linked glycans from all plant extracts examined. Furthermore, in collaboration, the presence of core α 1,3-fucose and its role in IgE binding was proven on a number of plant allergens, specifically, the Api g 5 allergen from celery, the Cup a 1 allergen from cypress pollen and the Cit s 1 orange allergen. The widespread occurrence of this sugar modification in plants correlates with immunological cross-reactivities. One model for these are the antibodies raised against the plant glycoprotein horseradish peroxidase, which also contains core α 1,3-fucose.



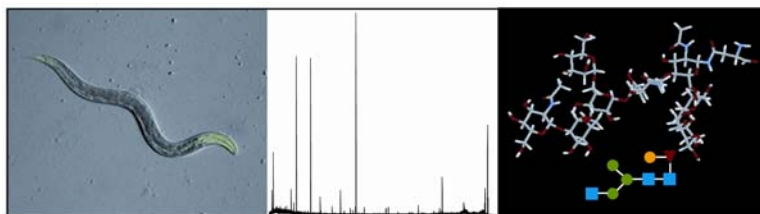
Data from the analysis of the molecular structure and antibody-binding characteristics of an orange allergen

Antibodies to horseradish peroxidase do not just recognise plant glycoproteins; it has been known for some twenty-five years that these antibodies can be used as a reagent to stain neural tissue in the fruitfly *Drosophila melanogaster* and other insects, but there was neither data as to the genetic origin of this staining. Since core α 1,3-fucose was previously found in the honeybee, I hypothesised that this modification would be also present in the fruitfly neural system. By use of complementary approaches (specific inhibition of anti-horseradish peroxidase staining in fruitfly embryos by core α 1,3-fucose-containing glycoconjugates, discovery of glycans containing this moiety and characterisation of a gene encoding a core α 1,3-fucosyltransferase enzyme capable of creating this epitope, use of RNA interference in a neural cell line), we succeeded in molecularly characterising this epitope in *Drosophila* and determined its genetic origin.



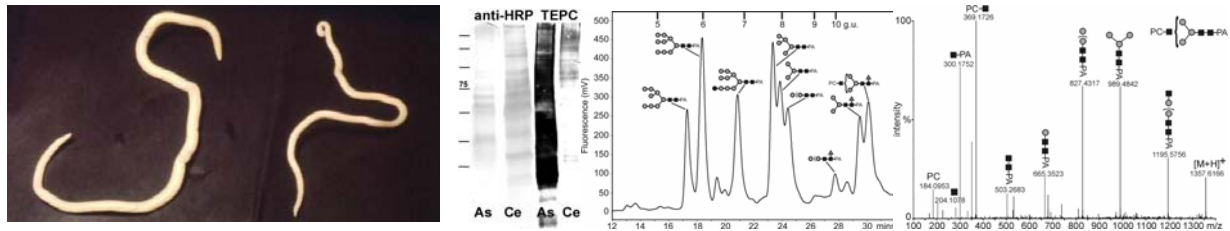
The neural expression of a core fucosyltransferase in fly embryos of different stages (the latter ones showing presence of the mRNA) and the reduced expression of anti-horseradish peroxidase epitopes in an *Drosophila* neural cell line treated with relevant double-stranded RNA, as shown by flow cytometry

Another model organism expressing anti-horseradish peroxidase epitopes is the nematode *Caenorhabditis elegans*. In this organism, the situation was more complicated than in the fruitfly since there were more candidate fucosyltransferase genes and since the glycan spectra of wild-type worms were far more difficult to interpret: indeed, many of the N-glycans from the worm are yet to have their exact structures determined. However, using specific gene knock-outs we successfully identified the single core α 1,3-fucosyltransferase gene in the worm responsible for generation of core α 1,3-fucose and furthermore found that it has, for this class of enzyme, an unusual and unexpected exact substrate specificity. In order to characterise these complex N-glycosylation biosynthetic pathways in the worm more extensively, we also studied another core fucosyltransferase (a core α 1,6-fucosyltransferase), a processing Golgi glycosidase (α -mannosidase II) as well as hexosaminidases; cloning, expression, promoter analyses and mass spectrometry were major methods in these studies.



A worm expressing a green fluorescent protein reporter, a mass spectral analysis of a glycan and the molecular model of the corresponding glycan found in a hexosaminidase knock-out worm.

These studies are just a beginning: in order to see whether *C. elegans* is a potential model for parasitic nematode worms, further knowledge on nematode glycosylation is required in order to prove the validity of using *C. elegans* in this context. Thus, we have initiated studies on the analysis of N-glycans from parasitic nematodes of agricultural relevance, focussing on glycans containing the immunogenic core α 1,3-fucose and immunomodulatory/immunogenic phosphorylcholine elements. The first of these studies, on the porcine parasite *Ascaris suum*, has recently been published and involved extensive use of modern mass spectrometry techniques. One theory is that glycan-bound phosphorylcholine in nematodes or bacteria may play a role relevant to the 'hygiene hypothesis', which seeks to explain why autoimmune and allergenic diseases are more common in developed countries, where parasite and microbial infection levels are now low.



Analysis of glycans of Ascaris suum by Western blotting, chromatography and mass spectrometry

The studies focussing on fucose and its presence in plants, insects and worms have not been the sole theme of my recent work. Due to my building up (from scratch) the use of molecular biology with the glycobiology 'division' of my department, it became possible to study other various previously-uncharacterised glycosyltransferases: for instance, the α 1,4-GalNAc transferase required for the biosynthesis of novel glycolipids in the fruitfly was first identified in my group. Also, I initiated studies on the important modification of proteoglycan core proteins by xylose: the glycosaminoglycan chains of proteoglycans are important in regulating morphogenesis, growth factor gradients and also as part of heparin. The enzyme required for the initiation of such glycosaminoglycan chains is a xylosyltransferase; we characterised, in recombinant form, the worm, fly and human xylosyltransferases using new methods for analysing the enzyme products.

Currently-planned and ongoing work builds on the methodologies and systems used in the aforementioned studies. Four FWF projects deal with (i) the glycosylation of *Caenorhabditis elegans* and nematode parasites, (ii) the slime mould *Dictyostelium discoideum* (which also possesses anti-horseradish peroxidase epitopes and may be a model for pathogenic amoeba or for production of proteins from organisms with AT-rich genomes, such as the malaria parasite), (iii) re-engineering glycosylation in insect cells for the purposes of producing recombinant proteins, such as antibodies or erythropoietin, with 'optimised' glycosylation (e.g., lacking immunogenic fucose residues) and (iv) the amoebal parasite *Acanthamoeba castellanii*. Thus, the potential to exploit glycans from the immunological, medical and commercial perspectives are increasingly a focus of my work.