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**ABSTRACTS SUBMITTED FOR THE
24TH MEETING OF THE
SOCIETY FOR GLYCOBIOLOGY**

**November 23–26, 1996
Boston, Massachusetts**

17. Structure and Conformation

17.01

Conformational Stability of Recombinant Decorin and Biglycan:

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Biglycan (BGN) and decorin (DCN) belong to a family of small extracellular matrix proteoglycans characterized by a core protein that contains a leucine-rich repeat (LRR) domain. DCN and BGN consist of a core protein of ~42 kDa substituted with one or two glycosaminoglycan chains, respectively. DCN has three N-linked oligosaccharide attachment sites, two of which are conserved in the core protein of biglycan. The LRR-motif which has been conserved through out evolution is thought to be involved in protein-protein interactions. Studies demonstrating *in vitro* interactions of BGN and DCN with other matrix components have typically used proteoglycans extracted from tissues with chaotropic (4 M GuHCl) solvents which may irreversibly alter structural and functional domains.

We are using a biophysical approach to study the structural and functional domains of DCN and BGN. Quantitative amounts of these proteoglycans have been generated by transient eukaryotic expression in a human epithelial cell line (HT-1080) using the vaccinia virus/T7 bacteriophage system. In this study we have utilized intrinsic fluorescence and circular dichroism (CD) spectroscopy to determine the conformational stability of DCN and BGN. Fluorescence measurements were used to observe the changes in environment of endogenous tryptophan and tyrosine residues as a measure of changes in tertiary structure, while CD measurements at 222 nm were used to observe changes in overall secondary structure. The fluorescence emission maxima for native proteoglycan was at 342 nm, indicating a buried environment for the single Trp residue present in both DCN and BGN. In the presence of 6M GuHCl, the emission spectra was red-shifted to a lower energy state with a maxima at 352 nm, indicating the Trp residue is exposed to a polar milieu. The far-UV CD spectra of the native recombinant proteoglycans showed a minima at 218 nm, consistent with a secondary structure that is predominantly β -sheet (computer modeling prediction of 54% β -sheet, 14% β -turn, 12% α -helix, and 20% random coil). CD-spectra of proteoglycans extracted from bovine cartilage had a minima at 205 nm, indicating a loss in secondary structure. The equilibrium constant and the free energy change (ΔG) for the folded to unfolded transition was determined.

Differential scanning spectroscopy was also used to determine the thermal unfolding for these proteoglycans. Thermal denaturation curves reveal that the proteoglycans undergo a sharp transition around 50°C. The transition peak was asymmetric indicating a non two-state transition. The thermal unfolding was an irreversible process. Determining the stability curves for these proteoglycans will reveal additional features of these proteins such as the existence of functional domains, importance of glycosylation in their stability and the presence of stable intermediates.

(¹The first two authors contributed equally to this work)

17.02

Schizosaccharomyces pombe Produces Novel Pyruvate-Containing N-Linked Oligosaccharides; T. R. Gammill¹ and R. B. Trimble^{1,2}; ¹Department of Biomedical Sciences, State University of New York at Albany School of Public Health, and ²Wadsworth Center, New York State Department of Health, Albany, New York 12201-0509 (USA)

We have shown that *Schizosaccharomyces pombe* processes oligosaccharide-lipid and N-linked oligosaccharides in the conventional manner up to the Man₅GlcNAc₂ stage, and that this yeast lacks the endoplasmic reticulum Man₆- α -mannosidase found in all other organisms studied to date. *S. pombe* then directly elongates this Man₅GlcNAc₂ with Man and Gal into either small (Hex₁₀₋₁₁) "core" oligosaccharides or large (Hex₁₋₁₁) galactomannans. Nearly all of the large N-linked oligosaccharides released by endo- β -N-acetylglucosaminidase H from *S. pombe* glycoproteins bound to anion exchange resin. Analyses for the presence of non-carbohydrate functional groups revealed no phosphate, sulfate, or acetate, however, approximately six molecules of pyruvic acid per glycan molecule were found on 98% of the oligosaccharides. Pyruvate moieties were acetal- (ketal-) linked to galactose residues in the R configuration to carbons 4 and 6. This is the first report of pyruvate functional groups being attached to N-linked oligosaccharides in yeast and appears only to be the second documentation of this sugar modification in eukaryotes. Partial acid hydrolysis of the large galactomannans yielded pyruvylated Hex₁₋₇ species, whose linkages are currently being examined. (Supported in part by USPHS NIGMS grant GM23900 to RBT)

17.03

The Carbohydrate Composition of a Spider Silk: *Nephila clavipes* Dragline.

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The dragline silk of the golden orb weaver spider, *Nephila clavipes*, holds great promise as a highly versatile biomaterial. While far stronger than steel and unusually resistant to chemical degradation, dragline fibers maintain an extraordinary degree of elasticity, making them well-suited to a broad range of applications requiring high-performance materials.

Through lectin-binding studies, our lab previously found that the dragline silk of *N. clavipes* is composed of glycoprotein subunits. We report that in addition to these covalently-bound oligosaccharides, a carbohydrate polymer is non-covalently associated with the dragline fiber. GC-MS was used to examine this glucose-rich polymer, which contains traces of at least three other monosaccharides and has been found to account for 3-5% of the dragline weight. MALDI-TOF mass spectrometry is used to determine the size of the polymer, and additional characterization will be conducted to determine whether the polymer is a polysaccharide or a heavily-glycosylated polypeptide. In addition, enzymatic studies are being carried out to determine the nature of sugar linkages in the polymer.

17.04

THE CONFORMATION AND DYNAMICS OF A ¹³C UNIFORMLY ENRICHED FLEXIBLE POLYSACCHARIDE BY MULTIDIMENSIONAL HETERONUCLEAR NMR AND MOLECULAR MODELING Qiuwei Xu and C. Allen Bush; Department of Chemistry & Biochemistry, University of Maryland Baltimore County, Baltimore, MD 21228

A method for constructing conformational models of flexible complex polysaccharides based on NMR data and molecular modeling is applied to a polysaccharide which is a lectin binding receptor important in coaggregation of oral bacteria. The method involves uniform biosynthetic enrichment of the polysaccharide from *Streptococcus mitis* J22 with ¹³C which allows accurate measurements of heteronuclear coupling constants from a three-dimensional coupled HMQC-NOESY spectrum. The improved resolution of the 3-D spectrum also provides a large number of accurate values of NOE cross peak volumes in a decoupled HMQC-NOESY spectrum. While it was not possible to construct a model for the flexible polysaccharide directly from the NOE data, a model was successfully built from the coupling constant data. A simple average over a linear combination of low energy conformations was selected which matched the experimental ³J_{CH} data within experimental error. Simulation of the NOE data for this same combination of conformers gave excellent agreement with experimental NOESY data. Molecular dynamics trajectories both with and without coupling constant constraints do not represent the experimental NOE and ³J_{CH} data as well as the linear combination model. The dynamics of the polysaccharide were investigated by measurements of the longitudinal and the rotating frame relaxation rates and the heteronuclear nuclear Overhauser effects. The results show the polymer to be highly flexible with a hinge at the (1→6)-linked galactofuranoside residue. Since there is no generally accepted scheme for interpreting polysaccharide dynamics, several different methods of data analysis were used including a reduced spectral density function method as well as several different methods in which a series of isotropically decaying rotational correlation functions are assumed. The different analyses all show that there are differing amounts of internal motion in the different residues of the polysaccharide. One possible interpretation of the data, which uses an extended version of the model-free treatment indicates that picosecond motion is exhibited to a similar degree by all the residues in addition to a slower motion on the nanosecond time scale whose amplitude is greatest in the hinge region around the (1→6)-linked galactofuranoside residue in the polysaccharide.