S10.14

A Computer Graphics and Computational Study of the Receptor-Toxin Complex of GM1 and the Heat-Labile Enterotoxin from *E. coli*

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The recently published X-ray structure of the heat-labile enterotoxin from *E. coli* (1), and its complex with lactose (2), and the three-dimensional structure of the natural receptor GM1, derived from NMR (3) and molecular modeling, has enabled us to perform docking experiments using computer graphics in combination with energy minimization and molecular dynamics. A description of the binding surfaces and important binding characteristics, such as hydrophobic and hydrogen bond interactions, will be given. Only minor differences in conformation were found between the complexed molecules and the respective free molecules. Comparative docking experiments using various isoreceptors, for which binding data and three-dimensional models exist ((4) and references therein), explain the variation in toxin affinity found for these structures.

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S10.15

Exocellular Glycopolymer of Corynebacterium Michiganense: Structure and Biological Function

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It was shown that Corynebacterium michiganense produced exocellular glycopolymer, purification of which was performed by ion-exchange chromatography on DEAE-TSK gel. Investigated glycopolymer gave on hydrolysis only one neutral monosaccharide, glucose, and as acidic component-hexuronic acid. The structure of carbohydrate moiety of glycopolymer was studied by nuclear magnetic resonance spectroscopy (^{13}C- and ^{1}H-), periodate oxidation, methylation analysis. It was characterized by regular structure with disaccharide repeating unit composed of one residue of glucopyranose and one residue of glucuronic acid connected by β-(1→4)-linkage:

→3)-β-D-GlcpA-(1→4)-β-D-Glcp-(1→

It's known the bacterial glycopolymers affect the immune system of macroorganism. As a result of studies we established the investigated glycopolymer is a high effective inductor of cytokines production such as tumour necrosis factor, interleukin-1 and -interferon. These properties of glycopolymer investigated may be used in development of medical means with diseases caused by viral and bacterial infections.

S10.16

The Development of a Chitin Assay for the Quantification of Fungus

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The accurate evaluation of *in vivo* drug sensitivity of fungi will facilitate development of effective treatment for fungal keratitis. Currently fungal presence is quantified by estimating the recovery of viable organisms from the infected cornea. Chitin, a structural polysaccharide (β1→4 linked *N*-acetyl glucosamine) produced by fungi, but not mammals, provides an alternative method for evaluating fungal mass within the cornea. We have developed an assay for chitin *in vivo* that is applicable to experimental keratomycosis. Chitin is first isolated by homogenizing the infected cornea and removing the soluble material with SDS at 100°C. This is then deacetylated with 21.4 M KOH at 130°C yielding the glucosamine polymer, chitosan, which is then hydrolyzed in 0.5 M H_2SO_4 at 100°C. The resulting glucosamine solution is treated with an equivalent volume of 5.5 M $NaNO_2$. This yields an HNO_2 solution which results in the deamination of the glucosamine to form 2,5-anhydro-D-mannose. Subsequent reduction of 2,5 anhydro-D-mannose with $NaB[^3H]_4$ yields 1-[^{3}H]-2, 5-anhydromannitol. This radio-labelled sugar is isolated by paper chromatography and quantified via liquid scintillation to provide an estimate of chitin content within the infected cornea. Preliminary results indicated that chitin content correlates with colony forming units recovered from infected corneas. The chitin assay shows promise as a specific and sensitive method to evaluate response to drug therapy *in vivo*. It may be useful in other types of fungal infections.

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S10.17

Identification of Specific Receptors in the Piglet Intestine for *Escherichia coli* K88 Fimbriae

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Enteric colibacillosis in the neonatal pig is frequently associated with enterotoxigenic *E. coli* which express the fimbrial adhesins K88ab, K88ac and K88ad. These fimbriae mediate adhesion of the bacteria to glycoconjugates in the intestinal epithelium of the piglet.

K88ac subunits have been purified and labelled via amino groups using digoxigenin-3-*O*-methylcarbonyl-ε-aminocaproic acid-*N*-hydroxy-succinimide ester and have been used to identify a specific receptor in piglet brush border membranes. When membrane glycoproteins were subjected to SDS-PAGE/Western blotting and probed with digoxigenylated K88, a receptor-active band of 200 kDa was revealed. *O*-glycosyl moieties were implicated in the binding of the fimbrial adhesin. The presence of this glycoprotein was found to be dependent on the phenotype of the animal and on the site of the intestine sampled.