Candida albicans (β1-2)-Mannans: Unique Solution Conformation and the Immune Response to Their Glycoconjugates

Mark Nitz, Chang-Chun Ling, Albin Otter, Joanna Sadowska & David R. Bundle
Department of Chemistry, University of Alberta, Edmonton/Alberta, Canada T6G 2G2

Introduction
The pathogenic yeast, Candida albicans, is increasingly difficult to treat due to resistance against known anti-fungal drugs. Vaccines based on the immunogenic (β1-2)-mannan of the C. albicans cell wall may offer an alternative treatment. NMR and immunochemical data presented below provide evidence that a surprisingly small epitope may be a candidate for creating a viable conjugate vaccine. The synthetic di- to hexasaccharides 1-5 were studied by NMR (Figure 1) and a detailed conformational analysis for pentasaccharide 4 is presented here.

Experimental NMR
NOEs based on 800 MHz TROESY [1] experiments in D2O at 30.0°C with a mixing time of 400 msec. NOE quantification based on the average of five β-Man-H1:H5 interactions set to a reference distance of 2.4 Å (from X-ray data). Calculations. Biosym Discover using the AMBER forcefield with exo-anomeric potentials; ring geometries enforced by 7-fold increased ring torsional energy terms; 5 nsec Molecular Dynamics (MD) run at 300K on the annealed and minimized structure.

Results
NOE-based distances for pentasaccharide 4 are shown in Figure 2 with MD calculated average distances, in parentheses. Other oligosaccharides exhibited similar n→(n+1), n→(n+2) and n→(n+3) effects. In addition to the typical inter-residue NOE, we observed and quantified a total of 12 NOE interactions (3 per sugar pair). These NOEs and associated inter-proton distances can only be reconciled with a highly organized, helical conformation (Figure 3) with a 3 to 4 residue repeat.

Immunochemistry of the C. albicans β-mannan
Di- and trisaccharides 1 and 2 are significantly better inhibitors than larger tetra- to hexasaccharides (3-5) with two monoclonal antibodies (MAbs) (Figure 4). Both MAbs confer protection in mouse models of infection. This size specificity contrasts with the typical pattern, where activity steadily increases and plateaus at about the size of a hexamer [2]. Our immunochemical findings correlate with the ordered conformation of the β1,2-linked manno- and suggest the design of simple anti-C. albicans conjugate vaccines. Preliminary data substantiate the proposition [3] that short oligosaccharides coupled to protein can provide an antigen capable of inducing protection against C. albicans. After three injections with trisaccharide 2 conjugated to BSA, a strong antibody response specific for the C. albicans β-mannan was detected (Figure 5).

Conclusions
- The helical propensity of β1,2-linked manno- and suggests the design of simple anti-C. albicans conjugate vaccines. Preliminary data substantiate the proposition [3] that short oligosaccharides coupled to protein can provide an antigen capable of inducing protection against C. albicans. After three injections with trisaccharide 2 conjugated to BSA, a strong antibody response specific for the C. albicans β-mannan was detected (Figure 5).

References

Acknowledgments
The National High Field Nuclear Magnetic Resonance Center (NANUC, Edmonton) for access to the 800 MHz spectrometer. This work was funded by NSERC, CIHR, AHFMR and the University of Alberta.