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

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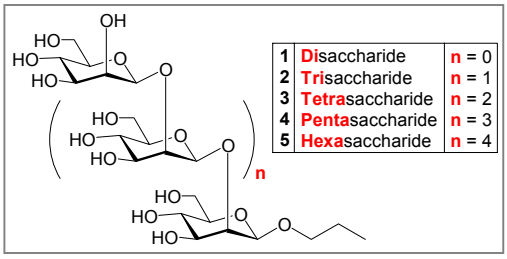
Introduction The pathogenic yeast, *Candida albicans*, is increasingly difficult to treat due to resistance against known anti-fungal drugs. Vaccines based on the immunogenic (β1-2)-mannan of the *C. albicans* cell wall may offer an alternative treatment. NMR and immunochemical data presented below provide 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 D_2O 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.

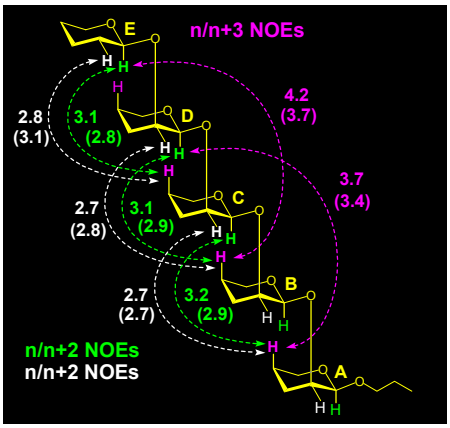


Figure 2 NMR-derived and calculated distances.

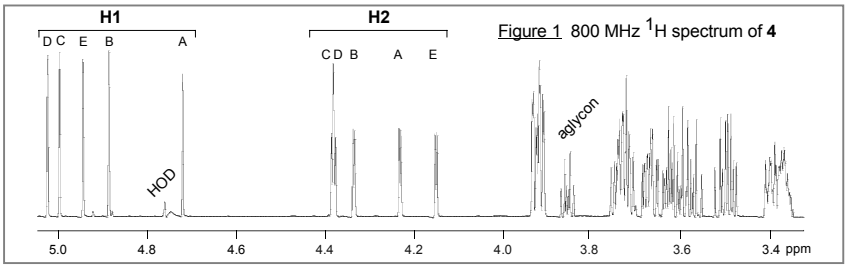


Figure 1 800 MHz ^{1}H spectrum of **4**

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.

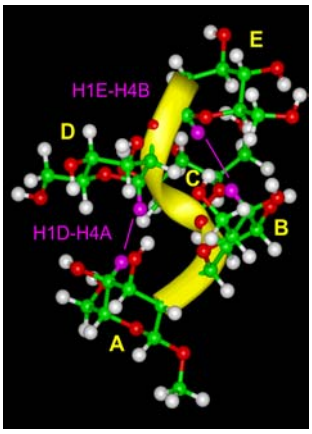


Figure 3 Computer-generated global minimum structure of **4** consistent with NMR data.

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 mannopyranans 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 mannopyranans creates a small epitope that is recognized by MAbs that are protective
- A trisaccharide conjugate vaccine induces polyclonal antibodies specific for the protective β-mannan cell wall antigen
- A conjugate vaccine may protect individuals at risk of developing serious conditions due to *C. albicans*.

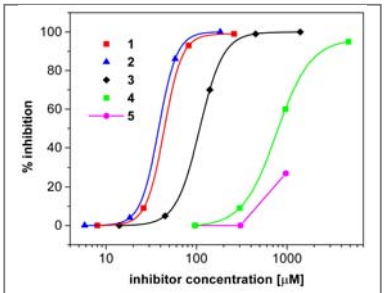


Figure 4 Synthetic oligosaccharides inhibit IgM monoclonal antibody B6.1 [4] binding to *C. albicans* cell wall mannan extract.

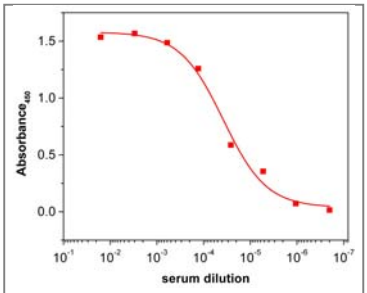


Figure 5 Trisaccharide **2** coupled to BSA was used to immunize rabbits. Titration of anti sera on *C. albicans* mannan coated ELISA plates.

References **1**. T.-L. Hwang & A. J. Shaka, *J. Magn. Res. B* **102**, 155 (1993); **2**. E. A. Kabat, *Federation Proc.* **21**, 694 (1962); *J. Immunol.* **97**, 1 (1966); **3**. M. Nitz, C-C. Ling, A. Otter, J. E. Cutler & D. R. Bundle, *J. Biol. Chem.* **277**, 3440 (2002); **4**. Y. Han, Y. & J. E. Cutler, *Infect. Immun.* **63**, 2714 (1995); *J. Infect. Dis.* **175**, 1169 (1997).

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