UNIVERSITEIT GENT

Department of Organic Chemistry

How does it stimulate? Understanding molecular recognition of peptidoglycan immunomodulators.

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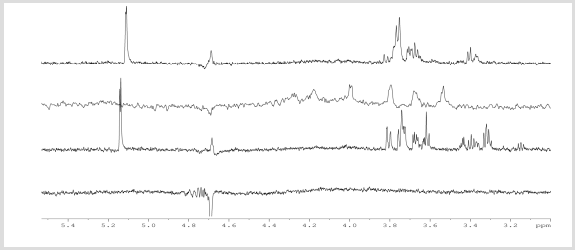
Introduction

Peptidoglycans (**PGN**) are constituents of the bacterial cell-wall consisting of large polysaccharide chains and short peptide units. PGN's are capable of triggering the body's immune defenses when recognized through intermolecular interaction by Pattern Recognition Receptors of the immune system. In this project we study the molecular recognition of multiple compounds (ATP, glucose, N-acetyl-D-glucosamine and a mannosylated adamantyl peptidoglycan derivative (**4a**[1])) by a model receptor, Concanavalin A (**ConA**). Binding epitopes and the strength of binding have been determined using Saturation Transfer Difference experiments combined with 1D and 2D NMR. All spectra were obtained using a 700 MHz NMR spectrometer (Bruker).

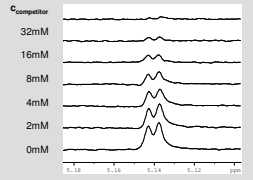
1. Saturation Transfer Difference (STD)

STD[2] is a NMR technique that allows to see the part of the molecule involved in intermolecular interaction with the receptor. The STD signal is more intense for protons closer to the binding site, which makes it possible to eventually map the binding epitope (Group Epitope Mapping, GEM).

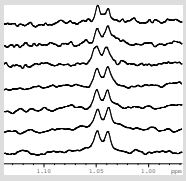


2. Competitive Titration

Competition titration is achieved by adding a competitor (methyl-α-D-glucoside) to the sample. The competitor binds stronger to the protein than the ligand, so the ligand is removed from the binding site and the intensity of the ligand STD signals decreases as can be seen for glucose. From the binding isotherm, the dissociation constant (K_d) of the binding can be determined[3].

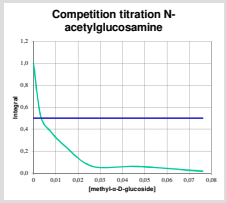


Competitive binding of glucose



Non-competitive binding of 4a

During the competition titration of 4a, it became clear that the ligand STD signals did not decrease, while the competitor STD signals did increase. This leads to the conclusion that 4a does not bind to the same binding site as the competitor.

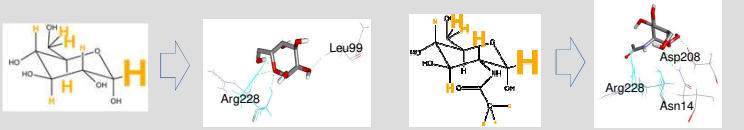


$$K_{a,lig} = \frac{[Lig]K_{D,comp}}{IC_{50} - K_{D,comp}}$$

	Kd (mM)
Glucose	1.5
N-acetyl-glucosamine	1.3

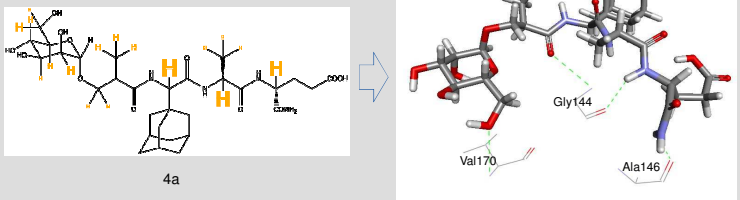
3. Group Epitope Mapping and Docking

The theoretical optimal docking positions were determined using AutoDock Vina[4]. These conformations were then compared with the results of the GEM, to confirm the actual binding epitope of the molecule.



α-glucose

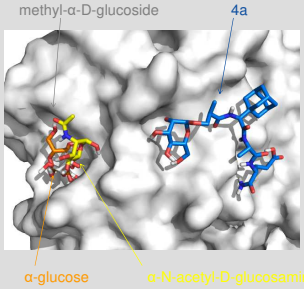
α-N-acetyl-D-glucosamine



4a

4a forms H bonds with the side chains of the residues near the competitor binding site.

Conclusions



- 4a binds weakly to ConA as evidenced by the STD effect.
- Competition experiments indicated that the binding site for 4a is different than that of methyl-α-D-glucoside. Due to the lack of competition, the K_d of 4a could not be determined.
- STD GEM showed that the ligand binding epitope involves the mannosyl group and the peptide backbone.
- Docking calculations indicate that residues near the known binding site are involved in the binding primarily by forming H bonds.

Safety issues

There are two major safety issues one should regard to whilst working in an NMR laboratory: magnetic and cryogenic safety.

The magnet contains the cryogenic liquids helium and nitrogen. During the refill of the magnet, which is only allowed to be done by trained personnel, one should always wear gloves, safety goggles and a lab coat. The oxygen level in the room can drop rapidly because of a magnet quench and subsequent release of evaporation gases. Therefore, the oxygen level of the room should be monitored and the room must be properly ventilated.

The magnet is surrounded in all directions by a strong magnetic field, which attracts ferromagnetic objects. Hence all equipment placed and used near the magnet should be made of non-ferromagnetic materials. Mechanical watches and pacemakers are also not allowed, since they could be damaged. The data on data storage devices could also be removed by the magnetic field.

In the room, the 10 mT line is marked, after which the strength of the magnetic field increases rapidly; one should be careful when crossing the line.

[1] Ribic, R., et al. (2012). "Influence of Mannosylation on Immunostimulating Activity of Adamant-1-yl Tripeptide." Chemistry & Biodiversity 9(7): 1373-1381.
[2] Mayer, M. and B. Meyer (1999). "Characterization of ligand binding by saturation transfer difference NMR spectroscopy." Angewandte Chemie-International Edition 38(12): 1784-1788.
[3] Cheng, Y. and W. H. Prusoff (1973). "Relationship between Inhibition Constant (K1) and Concentration of Inhibitor Which Causes 50 Per Cent Inhibition (I50) of an Enzymatic-Reaction." Biochemical Pharmacology 22(23): 3099-3108.
[4] Trott, O. and A. J. Olson (2010). "Software News and Update AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading." Journal of Computational Chemistry 31(2): 455-461.