Laboratory for Natural Product Chemistry

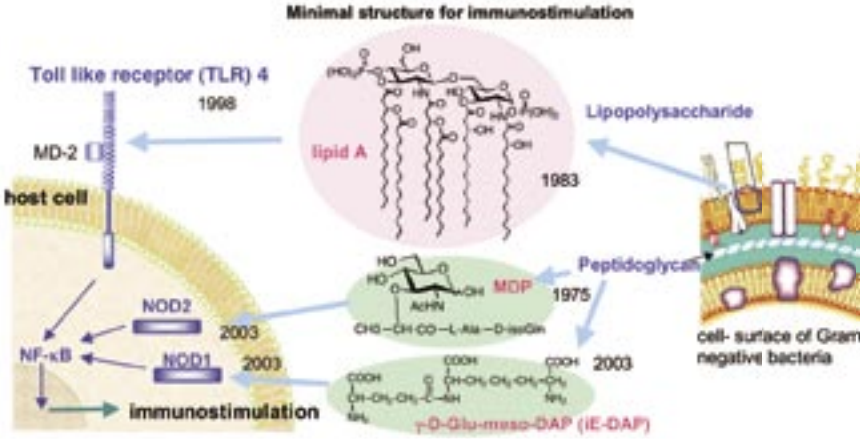
Professor: Koichi Fukase, Associate Professor: Yukari Fujimoto, Assistant Professor: Katsunori Tanaka

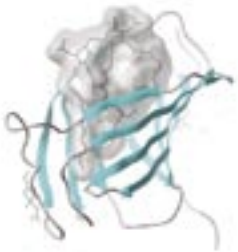
URL: http://www.chem.sci.osaka-u.ac.jp/lab/fukase/index.html
E-mail: koichi@chem.sci.osaka-u.ac.jp

The Biofunction of Bacterial Glycoconjugates in Innate Immunity

Innate immunity is a phylogenetically ancient defense mechanism against microbes. The recognition by innate immune receptors of molecules specific to microbes is a fundamental process in innate immunity. Bacterial cell-surface glycoconjugates such as lipopolysaccharides (LPS) and peptidoglycans (PGN) are known to be strong immunopotentiators. Our laboratory has demonstrated that the active principle of an LPS is its lipid part (lipid A), and the minimal active structure in a PGN is muramyl dipeptide (MDP).

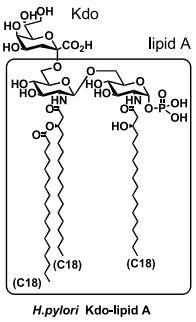
LPS is a cell surface glycoconjugate found in Gram-negative bacteria. Various structural analogues, including radio- and fluorescence-labeled derivatives, have been synthesized to elucidate the mechanism of immunostimulation. Recognition of lipid A by the LPS receptor, a complex of toll-like receptor 4 (TLR4) and its association protein MD-2, was observed by using these synthetic probes. X-ray crystallographic analysis of co-crystals of MD-2 with tetra-acylated lipid A showed that MD-2 plays a principal role in LPS recognition.





Crystal Structure of Human MD-2 with tetraacylated lipid A (lipid VIa)

LPS of *Helicobacter pylori*, often found in patients with chronic gastritis, shows much lower activity than *Escherichia coli* LPS. We synthesized the LPS partial structures of *H. pylori* and found that both *H. pylori* Kdo-lipid A and the lipid A part showed antagonistic activity. This suggests that the antagonistic effect of these LPS is one of the causal factors of their pathogenicity.



H.pylori Kdo-lipid A

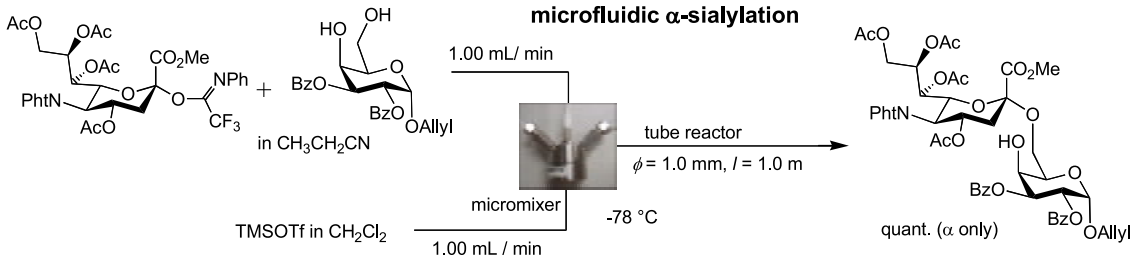
Peptidoglycan (PGN), a major component of cell walls, is a mesh of alternating β(1,4) linked muramyl-glucosaminyl glycans that are crosslinked by peptide chains. We have synthesized various PGN partial structures, i.e., mono-, di-, tetra- and octasaccharide fragments and found that the intracellular protein NOD2 recognized the partial structures containing the MDP moiety.

We showed that NOD1 recognized the dipeptide γ-D-glutamyl-*meso*-diaminopimelic acid (iE-DAP), which exists in Gram-negative bacterial PGN. Nod1 function *in vivo* has been investigated using the

potent Nod1 agonists that we have developed.

Efficient Synthesis of Oligosaccharides

Synthesis of oligosaccharides and glycoconjugates has played an important role in the elucidation of their biological functions. However, substantial time and effort is often needed. Extensive efforts have therefore been made to establish an efficient synthesis for oligosaccharides. To this end we have studied solid-phase and tag-assisted methods of oligosaccharide synthesis. The solid-phase synthesis of N-linked glycan in glycoproteins has been investigated to aid in the elucidation of their biofunctions. Our synthesis features the highly stereoselective β-mannosylation and microfluidic α-sialylation as well as (ii) the efficient glycosylation of the sugar units on polymer supports. Furthermore, (iii) a new radical-mediated deprotection of the *N*-trichloroethoxycarbonyl (Troc) group on the solid phase has been developed and successfully applied to the synthesis of N-linked glycans.



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