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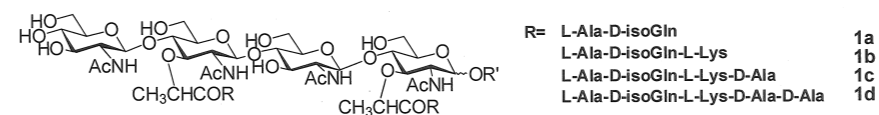
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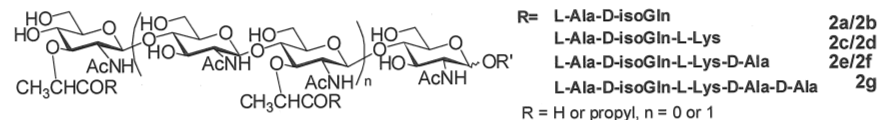
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### 論 文 内 容 の 要 旨

Peptidoglycan (PGN) is a major component of bacterial cell wall, consisting of glycans and peptide chains that form a three-dimensional mesh-like structure outside the plasma membrane. The glycan component consists of alternating residues of  $\beta$  (1 $\rightarrow$ 4) linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues, and are connected each other with peptide-linkages. Specific microbial molecules including PGN are known as the stimulating components of innate immune system, activating sensor proteins called pathogen-recognition receptors (PRRs). PGN is also known as the target of antibiotics, and also other enzymes and lectins.

Recent studies revealed that several protein families recognize distinct PGN partial structures at various different positions. There was a great deal of effort for the chemical synthesis of PGN fragments in the last decade in order to obtain the pure and discrete species for precise structural and biochemical studies. In the previous work of our group, nucleotide-binding oligomerization domain protein 1 (Nod1) and 2 (Nod2) were identified as the innate immune receptors of PGN. However, the comprehensive analysis of the substrate structures recognized by PGN receptors and other PGN recognition proteins using complex PGN fragments has not been well-conducted. In order to understand the recognition and functions of bacterial peptidoglycan with host multi-cellular organisms and also in the bacteria themselves, the library of PGN fragments was synthesized in this study. The library included two kinds of sequences of alternating glycans (GlcNAc/MurNAc), with various length of glycan chains. The targets covered the compounds having disaccharide, tetrasaccharide and octasaccharide.





Peptidoglycan fragments structures.

The synthetic targets 2b-h (Figure 1), which are the fragments expected to be produced by bacterial enzymes releasing to the environment, have not been chemically synthesized. The previously synthesized fragments 1a-d (in our group), which are considered to be cleaved by the enzymes in animals were also prepared for comparison in this research. The Nod2 stimulating activity of each synthetic peptidoglycan fragment was then evaluated.

Construction of PGN arrays was then performed by attaching synthesized PGN fragments on a solid surface of the array platforms. In order to introduce the PGN fragments to the array, several linkage structures were examined, and finally a linker with terminal amine was successfully attached to the PGN fragments. The arrays could be used to detect the PGN's receptors and recognition proteins (eg. peptidoglycan recognition proteins (PGRP), enzymes and lectins) and measure the binding properties of these proteins.

Many proteins containing PGN recognition domain, such as LysM, recognize relatively longer glycan chains. For the analysis of the detailed recognition of LysM domain, especially with LysM containing enzyme AtlB and AtlC from *Enterococcus faecalis*, the tetrasaccharide and octasaccharide containing PGN structures were synthesized. In order to prepare various fragment structures having longer glycan chains, a new efficient synthetic pathway was investigated. Appropriately protected 4-*O*-Fmoc protected disaccharide was thus used as the key intermediate in this route. Synthesis of tetrasaccharide as well as the octasaccharide with heptapeptide was successfully achieved.

In this thesis, I succeeded in developing the synthetic methods for PGN having relative longer glycans. The newly developed synthesis of PGN also enabled me to build the PGN fragment library, to form array of the library compounds, and also to investigate further study on the PGN functions including the recognition of LysM domain. This detailed chemical and structural study would contribute for understanding of bacterial immune stimulation and also for application to prevent the bacterial infection, with recognizing PGN structures.

## 論文審査の結果の要旨

王寧は“Synthesis of peptidoglycan fragment library for analysis of receptor recognition (認識タンパク質探索・解析を目指したペプチドグリカン・フラグメントライブラリ構築)”という研究題目で以下の研究を実施した。

王は、免疫増強複合糖質としてよく知られている細菌細胞壁ペプチドグリカンについて、種々のフラグメントのライブラリを化学的に構築し、それらを用いて、ペプチドグリカンを認識する様々なタンパク質との相互作用を解析した。

まず細菌由来酵素の消化によって得られると予想される新規なフラグメントを含めた 2 糖ならびに 4 糖ペプチド・フラグメント群の効率的な合成に成功し、それらを用いて自然免疫受容体 Nod2 の活性化のための新しい構造要因を見出した。

続いてペプチドグリカンフラグメントを結合させたマイクロアレイの作成に成功し、WGA レクチンとペプチドグリカン認識タンパク質 S (PGRP-S) との相互作用解析を行った。得られたマイクロアレイは動物由来の種々のペプチドグリカン認識タンパク質、ペプチドグリカン認識部位 LysM ドメインを有する

細菌や植物タンパク質群の相互作用解析研究に有用である。

また王は NMR を用いた LysM ドメインとの相互作用解析のために、4 糖ならびに 8 糖ヘプタペプチドの合成に成功した。ここで新しい戦略に基づくペプチドグリカンフラグメントの新規合成法を確立した。この研究において、ペプチドグリカンフラグメントが自己凝集を起こすことが見出された。この結果は天然ペプチドグリカンにおいても自己凝集により強固な分子構造を形成することを示唆するものであり、今後のペプチドグリカンの三次構造研究に新しい道を拓いた。

以上のように王寧は、糖質化学ならびに自然免疫分野の発展に大きく貢献した。よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。