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研究課題名（和文）βーグルカン構造に基づく新規ワクチンアジュバントの開発

研究課題名（英文）DEVELOPING NOVEL VACCINE ADJUVANTS BASED ON BETA-GLUCAN

研究代表者

ファイナ　ガルシア・マーティン（Garcia-Martin, Fayna）

北海道大学・先端生命科学研究科（研究院）・助教

研究者番号：３０５３７０８０

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研究成果の概要（和文）：本研究の最終目的は免疫学的応用が可能な新しいグルカン誘導体を開発することである。助成を受けた期間中は主にβ-グルカンを基に本研究を行った。
最初に1,3-β-グルカンを基に新しい種類のβ-グルカン開発を行った。その結果免疫システムは3重らせん型の長いグルカンを認識することが判明した。数回の実験後、複合糖質を合成に成功し現在その構造的そして生物的アッセイを行っている。β-グルカンを他の生物高分子と複合させるため、低酸性条件で高速そして選択的に反応を起こすグライコブロッティング方法を選択した。
本研究は固形および液状化学合成、そして生物的評価を含んだ挑戦的研究であり、その研究にて良い結果を得た。

研究成果の概要（英文）：The final aim of the project is to develop novel glycan-derivatives with application on immunotherapy.
During this term, the main investigator was able to follow the project based on beta-glucan conjugates. First, we focus on the developing of novel conjugates based on 1,3-beta-glucans. Immune system is able to recognize long glucans forming a triple helix structure; the aim is to mimic the same structure by using shorter polymers of beta-glucans conjugated with peptides. After challenging several drawbacks, the glycoconjugates were finally synthesized, finally structural and biological assays are undergoing.
This is a very challenging project including chemical synthesis on solid-phase, solution and biological evaluation, we got successful results and further studies are still undergoing. We are very confident to get a potential candidate as an immune system modulator. Further, this findings and novel approaches open avenues for broader applications.

研究分野：　Chemical biology

キーワード：　Glucan　biopolymers　conjugation　chemical synthesis　binding assays

１．研究開始当初の背景

(1) β-glucans binds to Dectin-1 and complement receptor 3. Dectin-1 is a pattern recognition receptor located on the membrane of dendritic cells and macrophages, this receptor is essential to recognize and phagocytes fungal pathogens (Brown et al. Nature. 2001; Kratky et al. PNAS. 2011). The exact mechanism of β-glucan recognition by Dectin-1 and its role as possible adjuvant is still unknown.

(2) In the rapid development of new adjuvants, synthetic platform will allow a more rational approach to the optimization of next generation compounds possessing greater potency and low toxicity.

２．研究の目的

(1) We propose to identify appropriate ligands for glycan binding proteins and to develop a carbohydrate-based targeting of immune system receptors. Among the small molecules which can elicit an immune response are the β-1,3-D-glucans which stimulate antitumor and antimicrobial response by inducing the production of cytokines and chemokines.

(2) Development of glycoconjugated based biomolecules able to be recognized by immune system and boost an immune response.

３．研究の方法

(1) For the synthesis of the monosaccharides, solution phase was employed. For the elongation of the glucans, solid-phase synthesis was preferred. Characterization of the compounds was performed by NMR, RP-HPLC and MALDI-TOF mass techniques.

(2) For the docking studies, we employed bioinformatic tools: i) Maestro Software to describe the structure and conformation of the ligads; ii) Autodock for the docking between ligands and Dectin-1 and iii) Discovery Study software for the visualization.

(3) To check the affinity of the compounds with the receptor, we employed microarray binding assay and STD-NMR.

(4) For the conjugation of the glucans with peptides, glycoblotting method is preferred as it permits the linkage in mild acid conditions and protecting groups are not required.

４．研究成果

(1) Chemical approaches

The project put the focus on the development of novel cancer vaccines and biomarkers based on glyco-conjugates.
Our first approach was to obtain beta-glucan polymers using solid-phase methodology.

In the early steps of the project, we focused on the synthesis of the starting materials, finally the building blocks were obtained. As donour, thiphenol donors were selected to ensure regioselective glycosylation. As temporal protecting group, Fmoc was chosen to control bond formation at position 3. Bz group was chosen as permanent protecting group, as it is orthogonal to Fmoc deprotection conditions and it helps to promote the beta-linkage.

Next, we tried several attempts to get the polymers of oligosaccharides. The following of the synthesis on solid-phase is not direct as the compounds are linked to the resin. First, we developed a novel colorimetric test to verify the presence of free alcohol groups (after coupling of the saccharide, if colorimetric test is positive, no total coupling of the sugar was accomplished thus double coupling might be performed). After several trials an adapted protocol based on methyl red emerged as the best option for following the solid-phase elongation.

Next, we investigate the deprotection of Fmoc group under several conditions. Usually, at solid-phase peptide synthesis, the usual way to deprotect Fmoc is by using piperidine in dimethylformamide (DMF). For this study, the use of dichloromethane (DCM) is preferred, finally we chose the triethylamine in DCM as the most convenient method as in only 30 min we could observe total deprotection of Fmoc without migration of the protecting groups.

As solid support, we preferred a polystyrene based resin as it is robust and hydrophilic, which suits with our chemical reactions. After optimization of the best conditions for following the reaction and for deprotection, the next element of study is the selection of the type of linker at the solid support. First, we tried with chlorotrityl resin, but under glycosylation conditions, the products were cleaved. Next, the aldehyde resin to create a diacetal product, with this resin, it was possible to get until trisaccharide beta-glucan product.

Also, attempts using Wang resin were successful, however the loading of the resin was always very low. Finally, we could obtain four, five and six mer beta-glucans.

For the obtaining of peptides and peptides decorated with glycans, also solid-phase synthesis under microwave irradiation is preferred.

(2) Docking experiments

Docking and STD-NMR experiments were done in collaboration with Prof. Jesús Jiménez-Barbero and Dr. Ana Ardá. For the docking experiments we employed Maestro software, AutoDock and Discovery Studio for visualization. After fixing the angles of beta-glucan and minimize the structure at Maestro software, AutoDock was employed to elucidate the binding and docking between the Dectin-1 protein and the beta-glucan. As receptor, the crystal structure of the murine Dectin-1 (2BPE) was used. In this crystal structure, Dectin-1 appears as a dimer and the beta-glucan location is placed between both monomers. However, in nature, the receptor might not form a dimer and according to Adachi et al. binding site might be placed at the "W223—H221" region. Following these findings, we decided to do docking at the specific binding pocket. According to figure 1, at binding site only few saccharides can interact but they may follow a precise structure as triple helix beta-glucan.

(3) Binding assays with STD-NMR

As by docking assays we could confirm that only four saccharides can enter into the binding pocket, we tried binding affinity assays with our tetra, penta and hexa-saccharides by using STD-NMR. This technology allows detecting the interaction between a receptor and the ligand, while providing information of the binding at the atomic level. Even if only few glucans can join at the binding site, we could not detect the binding. The possible explanation is that a longer polymer is able to create a triple-structure different than the flexible shorter polymers.

(4) Present and Future prospects

Further efforts are put on creating a system that can create a triple helix structure with a shorter beta-glucan polymer. In this sense, we are now on the way to synthesize glycoconjugates based on peptides to mimic the triple alpha helix structure. To conjugate the short beta-glucans to the peptides, the

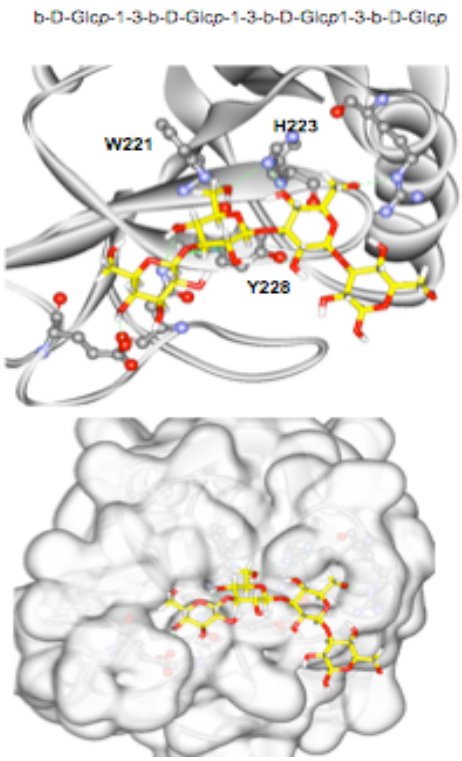

Figure 1. Docking of tetra-1,3-β-glucan ligand with monomer of Dectin-1.

glycoblotting method was chosen as it allows selective binding between the aldehyde of the reducing glycan and an oxime/hydrazide on mild acidic conditions without protecting groups. As a result, we could establish this novel protocol of conjugation between a polymeric dendrimer peptide and a beta-glucan. Further synthesis and binding assays are now under investigation.

Moreover, we could develop novel glycoconjugates based peptides with interest on cancer immunotherapy.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　7件）

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〔学会発表〕（計　5件）

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〔図書〕（計　0件）

〔産業財産権〕

○出願状況（計　0件）

○取得状況（計　0件）

６．研究組織

(1)研究代表者

Fayna Garcia-Martin
Hokkaido University, Graduate School of Life Science, Faculty of Advanced Life Science
Research Number: 30537080