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題目(和文)	糖修飾α-ヘリックスペプチドファージライブラリの構築と糖結合タンパク質リガンド探索
Title(English)	Phage display libraries of monosaccharide-modified α-helix peptides for carbohydrate-binding proteins
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種別(和文)	論文要旨
Type(English)	Summary

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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生物プロセス	系 コース	
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申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of （ 工学 ）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Carbohydrate-binding proteins (CBPs) presented on the cell surfaces are critical moderators in controlling biological systems, for example cellular communication networking and adhesion, tumor metastasis, inflammations and host-pathogen interactions. For these reasons, it is very much desirable to search for ligands that selectively engage to the binding domains of CBPs which could be further developed as potential sensing probes for diagnostic and therapeutic uses. Previously, our laboratory findings have demonstrated that a stable β-loop structure with monosaccharide modification was an important aspect of peptide-protein interaction and useful approach in designing good lectin ligands. Highly selective and strong ligands were identified with an affinity-based β-loop peptide phage display screening to different targets, including concanavalin A, insulin, and dihydrofolate reductase. Consequently, my studies were focus on another structural aspect, α-helix, for the construction of monosaccharide-modified peptides on phages, in order to expand the diversity of our glycopeptide library.

For my experiments, concanavalin A (ConA) and galectin-3 (Gal3) were chosen as model targets, because various aspects of the proteins physiochemical properties and their interactome have been well documented. Two 16-mer peptide phage libraries with tailored randomization were constructed by a genetic engineering method. The diversities of libraries were enough high and covered the theoretical diversity of clones. Phage library with three leucine residues was better for selection of CBPs, which was the optimized from the phage library with three alanine residues to improve the binding kinetics through extended hydrophobic forces. Before affinity selection rounds, phage-encoded peptides on pIII were chemically modified with thiol-bearing monosaccharide [mannose for ConA and galactose for Gal3] derivatives, by the formation of a disulfide bond to the thiol group of the cysteine residue. With the phage libraries generated, a few cycles of screening processes were performed for specifically bound phages to designated lectins. Unique individual clones were then analyzed via DNA sequencing and confirmed of their lectin-binding with phage enzyme-linked immunosorbent assay. Based on the screening, several clones were selected for further validation experiments with chemical methodologies such as circular dichroism and surface plasmon resonance, to assess the quality of peptides. All peptides formed helix structure as designed. Selective ligands with low dissociation constants were selected from the bio-panning method, in micromolar range for both target proteins.

In conclusion, I have successfully designed and constructed the monosaccharide-modified α-helix peptide phage libraries for lectins with fairly good sensitivity and specificity. Circular dichroism spectra revealed that engineering of peptides with a sugar unit did not interfere the ability to form α-helical structure. Furthermore, the findings demonstrated that the binding kinetics of monosaccharide-conjugated peptides had preferential binding to lectins because of the synergistic effects between the side chains of amino acids with the monosaccharide units. Thus, the methodologies and α-helical-constrained peptide phage library described here could be used for the selection of ligands with better specificity and improved affinity towards the corresponding lectins. Through synergistic effect, I believe that these structurally-constrained monosaccharide-modified peptides are useful to be developed as potent probes against their corresponding lectins. All glycopeptide ligands identified can be attached to the surface of nano/solid carriers, serving as targeting moieties for research, diagnostic and therapeutic uses.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
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