【Grant-in-Aid for Scientific Research (S)】

Broad Section E

Title of Project : New Synthetic Methods for Glycoproteins and the Integrated Approaches to Elucidate the Functions of Post-translational Modifications

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【Purpose and Background of the Research】

Glycoproteins have two types of glycans which are *N*-type and *O*-type sugar chains attached to the amide side chain of asparagine and the hydroxy group of serine/threonine, respectively. However, because these glycans show considerable heterogeneity in their structure, we can not specify which structure is essential for the individual biological event. In this research project, we will use homogeneous glycoprotein synthesized for the elucidation of glycan functions. We aim to establish an unprecedented method to synthesize glycoproteins within a few chemical conversion steps by coupling long peptides expressed in *E. coli* with glycosylated amino acids in an aqueous solution. In addition, we would demonstrate that glycans have functions such as constructing a unique hydration shell on protein surfaces like sugar syrup and promoting the interaction between glycoproteins and their receptor proteins. We would also investigate the function of the glycans by introducing the synthesized glycoproteins into organelles and cell surfaces. Furthermore, we aim to analyze the structure of the complexes of the glycoproteins synthesized and their receptors

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【Research Methods】

A novel method to synthesize target glycopolypeptides will be studied with glycosyl asparagine thioacid of which the α-carboxy group of asparagine is converted to the thioacid group. Glycosyl asparagine thioacid would be coupled at its both α-amino and carboxy sites with two peptides expressed in *E. coli* to yield a long glycopolypeptide. We will use this method to synthesize glycoproteins within a few conversion steps. First, a peptide expressed in *E. coli* is activated to a peptide-thioacid, which is then coupled with the glycosyl asparagine thioacid to synthesize the glycopeptide thioacid. The resultant glycopeptide thioacid is then coupled with a peptide having a β-mercaptoamino acid at its *N*-terminus. Finally, glycoproteins are synthesized by desulfurization of the β-mercapto group and folding process. For this strategy, we would synthesize various β-mercaptoamino acids. We also examine the synthesis of glycoproteins with *O*-linked glycans and Ubiquitin.

To prove the function of the hydration shells of glycans enhancing protein-protein interaction, we would analyze the accumulation of water around the glycans of glycoproteins by hydrogen-deuterium exchange mass spectrometry. We will also evaluate the relationship between the function of the hydration shell and the effect of glycan structure.

We would develop a method for the synthesis of transmembrane-glycoproteins on the surface of living cells. The intracellular and transmembrane peptides of the transmembrane-glycoprotein would be expressed on the cell surface. The transmembrane peptide would be coupled with an extracellular glycoprotein part by protein-protein coupling methods to generate the native transmembrane glycoprotein.

We also aim to develop a system for introducing synthetic glycoproteins into the Golgi apparatus of cells, allowing elongation of their glycans through the biosynthetic pathway. We will use this useful probe to understand biosynthetic pathway of glycans.

Furthermore, we would clarify the structure of the complex of folding sensor enzyme (UGGT) and misfolded glycoprotein by CryoEM, and elucidate what features of the misfolded glycoprotein would be recognized by UGGT.

【Expected Research Achievements and Scientific Significance】

Using our unprecedented synthetic method, we would synthesize various glycoproteins within a short time and can set synthetic homogeneous glycoproteins in the living cells for the study of the functions of glycans.

Although the researches of DNA and proteins have dramatically advanced, the researches on glycan functions have been gradually developing. This research project will considerably accelerate the elucidation of glycan function and its applications.

【Publications Relevant to the Project】

・ Y. Maki, R. Okamoto, M. Izumi, Y. Kajihara, Chemical Synthesis of an Erythropoietin Glycoform Having a Triantennary *N*-Glycan: Significant Change of Biological Activity of Glycoprotein by Addition of a Small Molecular Weight Trisaccharide. *J.Am.Chem.Soc.* 2020, 142, 20671. https://doi.org/10.1021/jacs.0c08719

・ M. Murakami, (5 additional authors), Y. Kajihara*, Chemical synthesis of erythropoietin glycoforms for insights into the relationship between glycosylation pattern and bioactivity, *Science Advances*, 2016, 2:e1500678, DOI: 10.1126/sciadv.1500678.

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