Abstract of Thesis

Name (. Chaitra Chandrashekar)	
Title	Chemical modification of recombinant peptides with hydrophilic protecting group for expanding semisynthesis of glycoproteins (糖タンパク質の半合成に向けた親水性保護基を利用する発現ペプチドの化学修飾)

Abstract of Thesis

Glycosylation of proteins is the major post translational modifications (PTMs) that occurs in eukaryotes. An efficient way to study their structural and functional properties is by synthesis of homogeneous glycoproteins by total chemical synthesis or by semisynthesis. Total chemical synthesis allows desired modifications of proteins at the atomic-level, while it has several drawbacks such as size limitation of polypeptide, low yield, expensive starting material and time consuming. Semisynthesis involves recombinant expression and chemical synthesis that reduces the number of chemical conversion steps and increases yield in less time.

In order to perform chemical synthesis, Native Chemical Ligation(NCL) is a critical method to couple glycopeptide and peptide together. NCL requires cysteine at the suitable position in their peptide backbone, but some glycoprotein targets don' t meet this requirement, thus limiting the use of NCL reaction. Recently, this limitation is overcome by using selenocysteine (Sec) or unnatural mercapto amino acids instead of Cys to perform NCL followed by subsequent deselenization/desulfurization.

However, these modifications cannot be applied to the expressed protein from *E. coli*, because specific modification at the *N*-terminal is difficult. Chemical modification of recombinant peptide can be performed only after its partial protection. The *tert*-butyloxycarbonyl (Boc) group is commonly used for partial protection which makes protected peptide hydrophobic. This causes solubility problems that influence the reactivity in many reactions that are to be performed in aqueous buffer conditions and also hinders efficient purification by reverse phase HPLC.

Therefore, I envisaged to establish a general semisynthetic strategy for the synthesis of glycoproteins by chemically modifying *N*-terminal of partially protected recombinant peptide using Sec to perform NCL. The major solubility problem of partially protected peptides using Boc group, is solved by replacing it with a hydrophilic carbohydrate-derived protecting group. The glucose- and galactose-derived protecting groups were used only for α-amino group protection. I wanted to extend this for efficient introduction of protecting groups to lysine side chain of recombinant peptide. Therefore, glucose-, galactose- and lactose- derived protecting groups were activated with imidazole and protection was optimized.

For establishing the strategy, a small model peptide 10 was obtained by *E. coli* expression as a fusion peptide 9 to SUMO fusion tag, which was cleaved by specific SUMO protease. The resultant desired short peptide 10 was partially protected with glucose- and lactose-derived protecting group. The increase in hydrophilicity of partially protected peptides was proved by comparing the retention time (t_R) of peptides in reverse phase HPLC using routine acidic solvent system and neutral solvent system. Later, glucose-protected peptide 12 was used to perform an efficient enzymatic cleavage under aqueous condition using TEV protease to obtain partially protected peptide with free *N*-terminal 15. This peptide

could then be specifically modified at the *N*-terminal with Sec to obtain 19. Using Sec is advantageous because Sec reacts more faster in NCL than Cys and moreover -SeH (selenol) can be selectively removed in the presence of -SH (thiol) groups. In the final step, all the protecting groups were removed to give unprotected *N*-terminal selenocysteine modified recombinant peptide 20 which can be readily used for NCL.

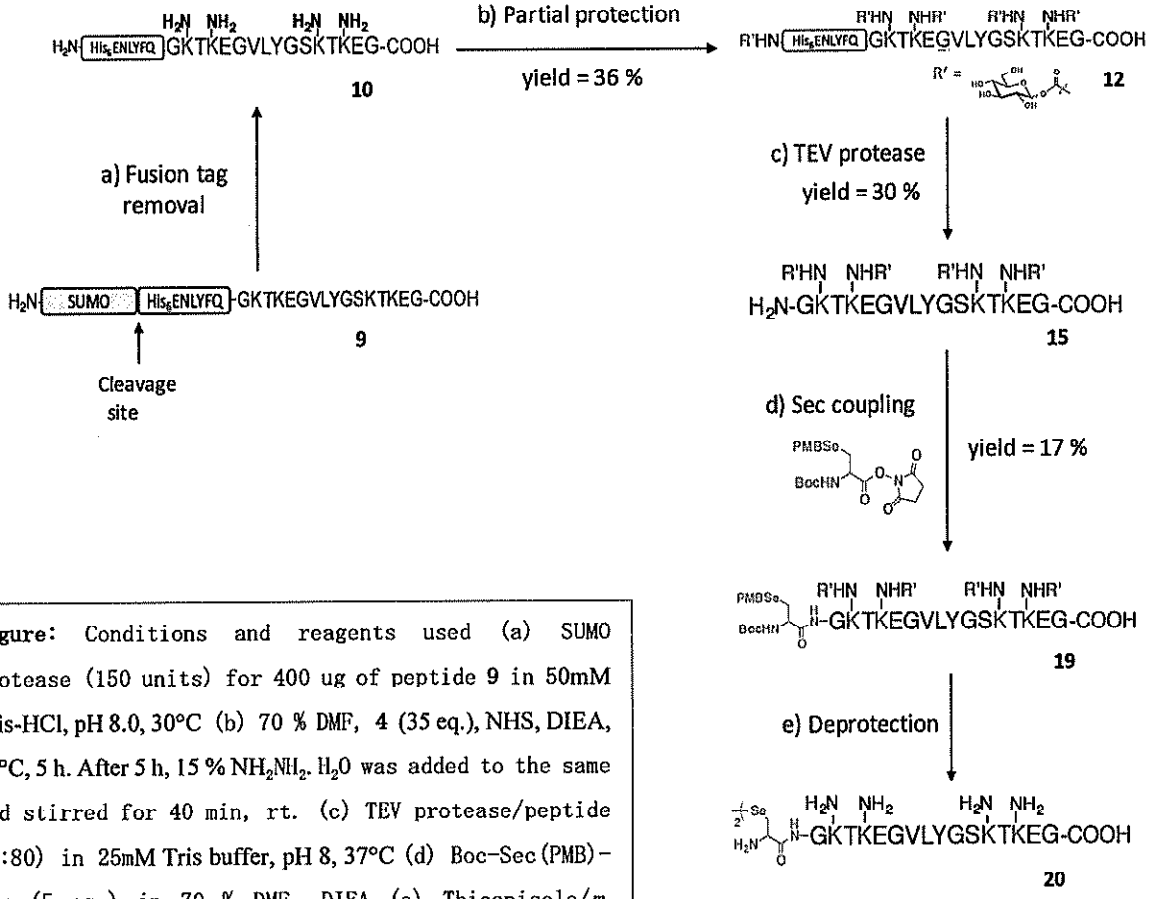

Figure: Conditions and reagents used (a) SUMO protease (150 units) for 400 ug of peptide 9 in 50mM Tris-HCl, pH 8.0, 30°C (b) 70 % DMF, 4 (35 eq.), NHS, DIEA, 40°C, 5 h. After 5 h, 15 % NH_2NH_2. H_2O was added to the same and stirred for 40 min, rt. (c) TEV protease/peptide (1:80) in 25mM Tris buffer, pH 8, 37°C (d) Boc-Sec(PMB)-OSu (5 eq.) in 70 % DMF, DIEA (e) Thioanisole/*m*-cresol/TFA/TfOH (2:1:20:2), 0°C, 2 h.

Thus, an efficient semisynthetic strategy was developed for the specific modification of N-terminal of recombinant peptide. By replacing the hydrophobic Boc-protecting group with a hydrophilic carbohydrate-derived urethane protecting group, I solved the critical solubility problem associated with current method of partial protection of recombinant peptide. This improvement allows access to various modifications of recombinant short peptides that was previously difficult. I could successfully install Sec which is an efficient functional group for NCL, the most critical peptide-peptide ligation strategy. This will advance the field of semisynthesis of various post-translationally modified proteins such as glycoproteins, phosphorylated proteins, and other desired modifications for structural and functional studies.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Chaitra Chandrashekar 氏は、“Chemical modification of recombinant peptides with hydrophilic protecting group for expanding semisynthesis of glycoproteins”という研究に取り組み以下の成果を上げた。

糖タンパク質の半合成法の確立のために、大腸菌発現系を用いたペプチドフラグメントの調製とそのN末端を活性化させ、糖アミノ酸等と連結するための手法の開発をおこなった。N末端にセレノシステインをもつペプチドは、native chemical ligation に利用できるとともに、水溶性フォスフィンで脱硫化しアラニンに容易に変換できる。そのため、ペプチド中のアラニンの位置で様々なペプチドが連結できる方法として注目されている。しかし、大腸菌等で発現したペプチドのN末端にセレノシステインを導入することはこれまで極めて困難であった。

本研究では、小型タンパク質であるSUMOフラグメントとTEVプロテアーゼが認識するペプチド配列を目的のフラグメントのN末端に結合させたものを大腸菌で発現する計画を立てた。このSUMO-TEV配列を含んだタンパク質断片を大腸菌発現系を用いて調製した後、SUMO protease を作用させて、SUMO配列を除いた後、N末端とリジン側鎖のアミノ基をグルコシルオキシカルボニル基で保護後、TEV protease を作用させた。これにより、TEVプロテアーゼが認識するN末端ペプチド領域を除去して、側鎖アミノ基が保護されたN末端遊離ペプチドフラグメントを得ることに成功した。さらに化学的に調製したペプチドも活用して、糖を用いたリジン側鎖の保護の条件検討、ならびにセレノシステイン残基の導入の条件を詳細に検討後、グルコシルオキシカルボニル基を酸により脱保護して、目的のN末端セレノシステイン含有ペプチドフラグメントを大腸菌発現と化学法を組み合わせて合成するための手法を確立した。

本研究において、用いるペプチドのリジン残基側鎖を糖残基を利用して保護することで水溶性を確保した保護ペプチドが調製できるようになったこと、そして、そのN末端を化学修飾できるようにしたことが評価される。また、その保護基には、グルコース、ガラクトース、そして二糖であるラクトースを用いることに成功した。この手法は今後広範に活用されることが期待できる。

上記の成果は、生命活動に必要不可欠な糖タンパク質の糖鎖機能を調べるための基礎的新規化学合成法として高く評価できる。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。