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A simplified procedure for gram-scale production of sialylglycopeptide (SGP) from egg yolks and subsequent semi-synthesis of $Man_3GlcNAc$ oxazoline

Bingyang Sun [a,b], Wenzheng Bao [b,c], Xiaobo Tian [b], Mingjing Li [b,c], Hong Liu [b], Jinhua Dong [a,*], Wei Huang [b,c,*]

[a] Key Laboratory of Structure-Based Drug Design and Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Pudong, Shanghai 201203, China
[c] iHuman Institute, ShanghaiTech University, Shanghai 201210, China

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ABSTRACT

Heterogeneity of glycan structures in native glycoconjugates always hampers precise studies on carbohydrate-involved biological functions. To construct homogeneous glycoconjugates from natural resource of homogeneous glycans is therefore a practical approach to solve this problem. We report here an optimized procedure for gram-scale production of sialylglycopeptide (SGP) containing a disialyl biantennary complex-type N-glycan from egg yolks. Our new procedure simplified the extraction process by treating the egg yolk powder with 40% acetone, avoiding massive emulsification, high-speed centrifugation, and sophisticated chromatography in reported methods. Subsequent semi-synthesis of the N-glycan core $Man_3GlcNAc$ oxazoline from SGP was accomplished for the first-time via glyco-trimming and successive oxazoline formation. This efficient semi-synthesis provides an alternative to the pure chemical approach that involves multi-step total synthesis and facilitates the application of *endo*-glycosidase-enabled chemoenzymatic synthesis of various homogeneous glycoconjugates.

1. Introduction

Glycosylation as a major post-translational modification of protein is involved in many important biological processes such as protein folding and quality control,[1–4] cell adhesion and migration,[5,6] viral entry and pathogenesis,[7,8] immune response and regulation,[9,10] etc. More than 50% of mammalian proteins and about 70% of clinical protein drugs are glycoproteins.[11] It has been tremendously demonstrated that glycan substructures may significantly affect functions of glycoproteins. However, heterogeneity of glycan structures in natural glycoproteins hampers the functional studies on precise structure–function relationship. As a solution to this problem, an efficient chemoenzymatic method for homogeneous glycoprotein synthesis has emerged, which takes advantage of the transglycosylation activity of *endo*-β-*N*-acetylglucosaminidases,[11–16] a class of endoglycosidases that hydrolyze the glycosidic bond in the *N*,*N*′-diacetylchitobiose motif of N-glycans, in the presence of glycan donor substrates.

Yamamoto et al.[17–19] reported the transglycosylation activity of *endo*-β-*N*-acetylglucosaminidase from *Mucor hiemalis* (Endo-M) using the egg-yolk sialylglycopeptide (SGP, Fig. 1)[20,21] as the donor substrate. The Endo-M enzyme cleaves the biantennary disialyl complex-type N-glycan from SGP and transfers it onto another GlcNAc-containing peptide/protein forming a new homogeneous glycopeptide/glycoprotein though in a relatively low yield. Thereafter, Shoda et al.[22] firstly discovered the synthetic glycan oxazoline, which is the transit-state form of *endo*-glycosidase-catalyzed N-glycan hydrolysis, could also serve as the transglycosylation donor substrate and dramatically improve the efficiency. Wang's and Fairbanks' groups synthesized a series of N-glycan oxazolines[23–32] including the core tetrasaccharide $Man_3GlcNAc$ oxazoline (Fig. 1) as the substrates of Endo-A from *Arthrobacter protophormiae*[33] that promoted excellent transglycosylation yields of resulting homogeneous glycopeptides and glycoproteins.

This chemoenzymatic transglycosylation approach presents a powerful tool for efficient synthesis of diverse glycoconjugates as summarized in Figure 1. Wang and his co-workers have made significant contributions to this method and successfully expanded its application in synthesis of important therapeutic glycoproteins,[34,35] glycopeptides[36–38], novel glyco-clusters,[39] glyco-natural products,[40] etc. (Fig. 1.) Recently, two papers have

* Corresponding authors. Tel.: +86 24 23986402; fax: +86 24 23904249 (J.D.); tel.: +86 21 20231000x2517; fax: +86 21 50807088 (W.H.).
E-mail addresses: dongjh66@hotmail.com (J. Dong), huangwei@simm.ac.cn (W. Huang).

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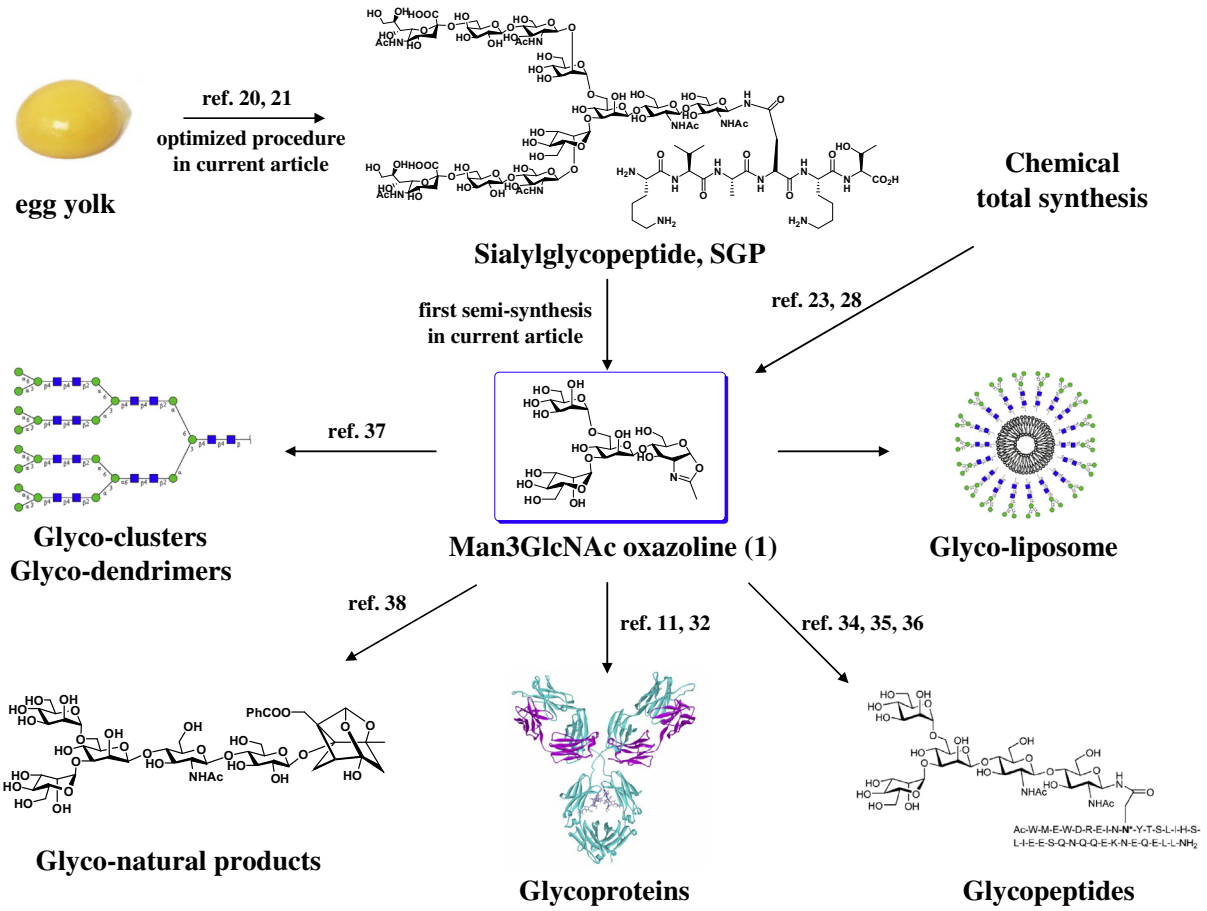

Figure 1. Preparation of N-glycan core Man3GlcNAc oxazoline and its application in chemoenzymatic synthesis of various glycoconjugates.

respectively reported the Fc-specific glycan remodeling of the monoclonal antibody drugs by Endo-S from *Streptococcus pyogenes* using $Man_3GlcNAc$ oxazoline as the homogeneous glycan donor.[11,34]

$Man_3GlcNAc$ oxazoline as the core N-glycan oxazoline has been employed in chemoenzymatic synthesis of various glycoconjugates (Fig. 1) and its chemical total synthesis has been reported by Wang's and Fairbanks' groups, respectively.[23,28] The total synthesis consists of over 30 synthetic steps with sophisticated process of regio-selective protection and deprotection, as well as stereo-selective glycosylation. The difficulty of long-step total synthesis in poor economic efficiency hinders the application of chemoenzymatic synthesis of glycoconjugates using the N-glycan oxazoline substrates. As an alternative option, semi-synthesis from natural homogeneous N-glycan resources was committed to prepare high-mannose type and biantennary complex-type N-glycan oxazolines in good yields.[41,42] Here, we report for the first time the expeditious semi-synthesis of core $Man_3GlcNAc$ oxazoline from the egg-yolk sialylglycopeptide (SGP) via enzymatic glyco-trimming and successive oxazoline formation reaction in a water solution.

SGP was firstly isolated by Seko et al. from egg yolks via phenol-extraction, centrifugal separation, ethylacetate wash, and comprehensive size-exclusion chromatography.[20] Zou et al. simplified the later-stage purification procedure using porous graphite carbon (PGC) extraction instead of reiterant gel-filtration operation.[21] However, the problem of emulsification in these methods caused a lot of trouble during all the processes before gel-filtration. Additionally, the requirement of long-time high-speed centrifugation for separation of phenol-extracting solution from precipitates limits the large-scale production. Sugawara et al. reported in a patent[43] using water to extract defatted egg yolks and treating the supernatant with alcohols or other organic solvents to precipitate SGP. But this method encounters the problems of emulsification, high-speed centrifugation, and complicated purification procedures as well. We developed a newly optimized procedure for gram-scale SGP preparation by reducing emulsification and averting centrifugation. This simplified method provides efficient scale-up of SGP extraction for potential industrial interest and facilitates expeditious semi-synthesis of N-glycan core $Man_3GlcNAc$ oxazoline for chemoenzymatic preparation of various glycoconjugates.

2. Results and discussion

2.1. Optimized procedure for gram-scale production of SGP

In Seko's approach, 9% phenol aqueous solution was employed to precipitate the unrelated proteins and extract the SGP from egg yolks. While, the tremendous emulsification occurred in the mixture probably is due to the blending of lipids, soluble proteins, organic solvent, and water. The extracts were highly emulsified so that separation of precipitates and supernatant was very difficult and incomplete even under high-speed (10,000*g*) centrifugation. A similar trouble also occurred when ethyl acetate was added to wash the supernatant after centrifugation. Thus, we sought to optimize the procedure to reduce or even avoid the emulsification for large-scale process.