



Glycosylation Of Hemicyanines An Overview

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ABSTRACT

The hemicyanin which has been found as very strong antibacterial activity. Hemicyanin acts as non-specific immune protein and acquires stronger antibacterial activity after glycosylation. The glycosylation of arthropod hemicyanin has been identified in *Haliothis tuberculata*. The diversity in glycan content and type of glycosylation of hemicyanin contribute to its functional properties and diversity. These modifications after the glycosylation of hemicyanin are also involved in pathogens recognition and bacterial invasion. The degree of glycosylation of hemicyanin subunits is largely affected by and depends on the presence of pathogens. Glycosylation plays an important role in infectious diseases. Many important interactions between pathogens and hosts involve their carbohydrate structures (glycans). Glycan interactions can mediate adhesion, recognition, invasion, and immune evasion of pathogens. In recent years changes in many protein N/O-linked glycosylation have been identified as biomarkers for the development of infectious diseases and cancers. The principal findings and the roles of glycosylation of both pathogens and host cells in the context of human important infectious diseases. Understanding the role and mechanism of glycan-lectin interaction between pathogens and host cell which may create a new paradigm for discovering novel glycan-based hemicyanine therapies that can lead to functional cure of pathogens infection.

INTRODUCTION

The hemicyanine dyes have been used in various purposes, e.g. photosensitizer, in space photography, as organic photoconductor for electrophotography, infrared absorbing material for optical recording system in the absorptivity and in semiconductor laser. The present invention relates about the glycosylation of respiratory protein hemicyanin. Hemicyanin is a copper-based glycoprotein that behaves as an oxygen carrier and is composed of a different number of subunits that assemble into an extremely large macro-molecular entity that exhibits a complex allosteric behaviour during oxygen binding. In present study four purified

hemicyanine EvH ,EvH 2,EvH 3 & EvH sub units from marine crab -Eriphia verrucosa hemolymph were glycosylated and several putative O-linkage sites were found in the identified amino acid sequence of EvH. Glycosylation is the combination of a carbohydrate with a hydroxyl or other functional group of another protein molecule to form a glycoconjugate. It is a form of co-translation and post-translation modification. Most proteins synthesized in the rough endoplasmic reticulum undergo glycosylation. Glycosylation is also present in the cytoplasm and nucleus as O-GlcNAC modification. Main Types of Glycosylation in Humans N-linked Glycosylation Many proteins are modified by N-glycosylation, which refers to the attachment of N-acetylglucosamine (GlcNAc) to the nitrogen atom of an Asn side chain by α β -1N linkage. These Asn-linked glycoconjugates contain a GlcNAc₂ mannose (Man)₃ core, to which a variable number of other monosaccharides can be added or removed. These additions include, for example, galactosylation, GlcNAcylation, sialylation, and fucosylation, and they determine whether the final structure is classed as a high-mannose N-glycan, a hybrid N-glycan, or a complex N-glycan. N-glycosylation depends on the formation of a lipid precursor. O-glycosylation can occur on amino acids with functional hydroxyl groups, which are most often Ser and Thr. In humans, the most common sugars linked to Ser or Thr are GlcNAc and N-acetylgalactosamine (GalNAc). GalNAc-linked glycans, often called mucin-type O-glycans, are abundant on many extracellular and secreted glycoproteins. Mucin-type O-glycan synthesis is initiated by polypeptide GalNAc transferases (GALNTs). Moreover, O-GlcNAc modification competes with protein phosphorylation at Ser and Thr residues, adding to the complexity of regulatory circuits. This functional role of O-glycosylation can be controlled by the expression levels of the enzymes involved, as well as substrate concentrations. The antibacterial effect of the hemicyanine isoforms as a consequence of the different glycosylation types in arthropods. The aim of present study was to investigate whether hemicyanine from E. verrucosa hemolymph and its glycosylated structural units have antibacterial effect against different clinical pathogens and whether its isoforms have potential for biotechnological application as antibiotics substitutes.

Functional diversity of Hemicyanine

protein and glycosylation differences between HMC-M and HMC-T antisera can contribute to the functional diversity of Hemicyanine. Hemicyanine is the major protein in shrimp sera, which bound which bound with bacteria and that HMC-M antigen possessed strong immune function. The in vivo antimicrobial activity of shrimp sera against E. coli strain which are ampicillin resistance, and the gene deleted mutant kanamycin resistance was further investigated. The rate of inhibition of shrimp sera against the deletion mutant of ompX, ompA was higher than that of the control. The ompW deletion mutant resulted in a decrease in the bacterial growth. These results suggest that the recognition of bacteria by the innate immune defence molecules. Hemicyanine doesn't always contribute to bacterial growth inhibition ability of HMC-M antigen but depends on other factors so as to exert an effect on multiple target.

The glycosylation is one of the most important post-translational modifications of Ig (immunoglobulin) to regulate its function, so we supposed that hemicyanine might also be glycosylated, especially HMC-M to regulate its functions. As compare the glycosylation of HMC-M to HMC-T by measuring the total amount of glycan, glycan components and glycan oxidation. The total glycan concentration was measured using

the phenol -sulfate acid method. A higher glycan concentration was found in HMC-M (3.0 to 0.05 %) than in HMC-T (1.46 to 0.19%). The difference in glycan components between HMC-M and HMC-T was determined using Tricine-SDS-PAGE, HPLC, lectin blot and FAR-Western blot analysis. The results from the Tricine-SDS-PAGE analysis revealed that the molecular weight of the main degraded fragments digested with Trypsin from HMC-M were significantly higher than those from HMC-T.

Observation

The degraded peptide were further analysed by HPCL. In particular, 67.6 and 8.6% peptides from HMC-M and HMC-T were detected at 3.5 minutes, while 8.4 and 76% were for at 35 minutes. The above observations from total glycan concentration determination, these findings suggest that HMC-M had higher glycosylation to HMC-T. The lectins including CON-A, recognising alpha-D-mannose is greater than alpha-D-glucose. These degraded peptides were further analyzed by HPLC. Approximately 10 and 24 peaks were observed in HMC-M and HMC-T, respectively. In particular, 67.6 and 8.6% peptides from HMC-M and HMC-T were detected at 3.6 min, while 8.4 and 76.1% were found at 34.9 min. Consistent with the above observations from total glycan concentration determination, these findings suggested that HMC-M had higher glycosylation compared to HMC-T. Moreover, lectins including CON-A (recognizing alpha-D-mannose > alpha-D-glucose), DBA (recognizing N-acetyl-D galactosamine), PNA (recognizing beta-D-gal-(1,3)-D-acetyl galactosamine), and UEA-1 (recognizing alpha-L-fucose) were used to blot the type of glycans in HMC-T and HMC-M. We observed that CON-A only bound the small subunit of both HMC-T and HMC-M, while the other three lectins could recognize the two subunits of them in a degree. Notably, both CON-A and DBA had more extensive staining with HMC-M than HMC-T, on the contrary, no difference between HMC-M and HMC-T with the PNA and UEA staining was found. This data indicates that HMC-M contains more alpha-D-mannose > alpha-D-glucose and N-acetyl-D galactosamine than HMC-T (1.21:1 and 1.54:1, respectively). Consistent with the Far-Dot-ELISA results, the relative integrated intensity in HMC-M was about 1.6 fold that of HMC-T. To further substantiate the glycosylation of HMC-M by CON-A and DBA, HMC-T and HMC-M were incubated with blotted lectin CON-A, and then reacted with anti-shrimp HMC-T antibodies. It was observed that there was a reduction in alpha-D-mannose > alpha-D-glucose but an increase in N-acetyl-D galactosamine in HMC-M. We then went on to investigate the effects of glycan free HMC on hemolytic activity. Both HMC-M and HMC-T were first subjected to the periodate oxidation assay, which results in the destruction of their carbohydrate epitopes. Interestingly, the hemolytic activity was completely abolished after deglycosylation by oxidization, as there was a drop from 89.41. to 0.93% for HMC-M and from 7.53 to 0% HMC-T. Thus, with these results on the glycosylation of HMC-M couple with the SNPs in the D3 domain it can be speculated that all these together might contribute to the functional diversity of hemicyanine.

Degree of Glycosylation

The degree of glycosylation of the hemicyanine subunits was of particular interest since hemicyanine samples were obtained from crabs inhabiting a microbially overloaded marine environment. The carbohydrate content of the five dissociated hemicyanine fractions (SUI-SU5) determined by the orcinol-sulphuric test. In this study, all structural units of *E. verrucosa* hemicyanine were glycosylated. The highest carbohydrate

content was found in fraction SUI (0.90 mg/mL), SU4 (0.30mg/mL), and the lowest carbohydrate content was in fraction SU3 (0.25 mg/ mL). The protein content of the fractions did not always correlate with the carbohydrate content. For example, fraction SU5 was more glycosylated than fractions SU3 and SU4, which had higher protein content. In earlier study showed that only three out of four isolated *E. verrucosa* hemicyanin subunits were glycosylated. No glycans were identified in subunit 4. In that investigation, the haemolymph was obtained from crabs inhabiting a different region. We speculate that the glycosylation diversity contributed to the functional diversity of hemicyanin and represented environmental adaptation to each habitat transition.

Conclusions

In this study, we report for the data about the glycosylation of hemicyanine and its antibacterial activity of *E. verrucosa* hemicyanin isoforms. The obtained results indicated that the degree of glycosylation of hemicyanin isoforms is likely determined by microbial contamination in the environment and strongly suggested that some subunits could be used as a platform for the discovery of novel antibacterial drugs. Future studies of the active domains of the hemicyanin subunits and direct induction of their glycosylation will allow the production of recombinant proteins with strong specific binding to clinical pathogens that have acquired resistance to conventional antibiotics.

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