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Commentary Article

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# Short commentary on Our Current Understanding of Red Blood Cell (RBC) Glycoproteins

**Takahiko Aoki**

*Department of Life Sciences, Mie University, Tsu, Japan*

## Introduction

In the Edo period of Japan, drinking carp blood was well known to the people as a folk remedy for tuberculosis. This bacteriostatic activity of blood is suggested to exist some antimicrobials in carp blood. We isolated a high-purity carp glycophorin (membrane protein containing sialo-oligosaccharide) from carp red blood cell (RBC) membranes using the lithium diiodosalicylate (LIS)-phenol method and streptomycin treatment.

The presence of glycophorins in RBC membranes of carp was observed on SDS electrophoresis gels by Coomassie blue and PAS staining [1]. While membrane proteins from carp membrane preparations are similar to those of human RBC membranes, carp RBC membranes showed different glycophorin patterns. The major glycophorin from carp membrane preparations is positioned near the human glycophorin A (dimer). According to the amino acid composition of carp glycophorin, there was no striking difference from human glycophorin A [1]. Although human glycophorins A and B carry the MN and Ss blood group antigens, it is unclear whether carp glycophorin carries those blood antigens, as no blood group antigen reaction has been observed by titration (unpublished materials).

## Commentary

The TLC analysis suggested that only the N-glycolylneuraminic acid (NeuGc) form of sialic acid was detected in the carp glycophorin. Using TLC, the hexosamine obtained from the carp glycophorin hydrolysate was identified as galactosamine [1].

The oligosaccharide fraction was prepared by β-elimination from glycophorin [2]. The oligosaccharide fraction was separated into two components (P-1 and P-2) using a Glyco-Pak DEAE column. These O-linked oligosaccharides (P-1 and P-2) were composed of glucose, galactose, fucose, N-acetylgalactosamine and NeuGc. The P-1 and P-2 contained one and two NeuGc residues, respectively. However, this method leads necessarily to contain salts in the obtained fraction. These P-1 and P-2 fractions were desalted using a GL-Pak Carbograph cartridge with ammonium bicarbonate in acetonitrile as an eluant [2].

To clarify the physiological activity of carp glycophorin, we performed the sensitivity test for the growth of several bacteria using the disk-plate method. These results suggested that the carp glycophorin exhibited bacteriostatic activity, and this activity is observed on all tested bacteria including three known fish pathogens (Gram-positive bacteria: Micrococcus luteus and Bacillus subtilis, Gram-negative bacteria: Vibrio anguillarum, Edwardsiella tarda, Aeromonas hydrophyla, Escherichia coli, and Pseudomonas fluorescens) [2,3]. Using the sensitivity test for the growth of several bacteria, fractions from the carp RBC membranes, the glycophorin oligosaccharide and P-1 also exhibited bacteriostatic activity; whereas the glycolipid fraction and the glycophorin fraction without sialic acid did not show the activity [2].

The efficacy of carp glycophorin as an antibiotic reagent was evaluated by the MIC values. These results suggested that carp glycophorin was effective against Gram-positive bacterium as same as oxolinic acid (OA) and miloxacin (MLX). In the case of Gram-negative bacterium, carp glycophorin was more effective than sulfamonomethoxin (SMM) [3].

In the blood of diseased carp infected by P. fluorescens, carp glycophorin is released from RBC membranes and interacts with the bacterium [3]. Under electron microscopic observations, the released carp molecule from the RBC attaches to the flagellum of V. anguillarum or the cell surface of M. luteus and inhibits bacterial growth [1]. The bacteriostatic activity of carp glycophorin is caused by the sialo-oligosaccharide (P-1 fraction) from carp glycophorin and is attributed to the nature of the lectin receptor. It is thought that some lectin-like proteins exist on the surface of Gram-positive bacteria or the flagellum of Gram-negative bacteria. These observations indicated that carp glycophorin is released from RBC membranes and adsorbed onto the surface of invading bacteria in the blood.

Using asialo P-1 fraction for NMR analysis and the permethylated P-1 for GC-MS, we determined that the structure of the bacteriostatic P-1 was NeuGcα2→6(Fucα1→4) (Glcα1→3)Galβ1→4GalNAc-ol [3]. The most commonly elucidated glycophorin oligosaccharide include the tetrasaccharide core containing N-acetylneuraminic acid (NeuAc): NeuAcα2→3Galβ1→3(NeuAcα2→6)GalNAc-ol and the trisaccharides: Gaβ1→3(NeuAcα2→6)GalNAc-ol and NeuAcα2→3Galβ1→3GalNAc-ol. A NeuGc-containing O-linked oligosaccharide has also been reported from horse, pig, goat and rabbit glycophorins, and the most commonly reported structure is a trisaccharide: Galβ1→3(NeuGcα2→6) GalNAc-ol.

P-1 oligosaccharide from carp glycophorin was unique for a vertebrate with respect to the hexosamine and hexose linkage and its non-chain structure. The 1→4 linkage of N-acetylgalactosamine is unique compared with other O-linked oligosaccharides of mammalian origin. Interestingly, the β1→3 glycosidic linkage of xylan, which is a component of the seaweed cell wall, is unlike the standard β1→4 linkage of land plants [3]. It is possible to detect the β1→4 linkage of N-acetylgalactosamine in marine organisms. From the NMR spectra, the characterized proton signals of the asialo P-1 fraction revealed an overall downfield shift in the resonance of αGlc and αFuc, except for the H-1 signals. This O-linked oligosaccharide indicates a non-chain-like structure unlike other glycophorin oligosaccharides [2].

***Corresponding author:** Aoki T, Department of Life Sciences, Mie University, Tsu, Japan, E-mail: aoki@bio.mie-u.ac.jp

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The purpose of our research on fish glycophorin is for practical applications as antimicrobials. The drawback for practical application is the large-scale preparation from fish blood. On this reason, we have developed the simple preparation method of carp GP with its high recovery for practical applications. This preparation method for application took a Japanese patent (Patent application: 2009-95745).

Recently, we performed the sensitivity test by using yellow tail glycophorin, another fish source preparation, for the growth of M. luteus by the disc-plate method. M. luteus formed inhibition zones around the paper disc containing the yellow tail glycophorin fraction. This result suggested that the yellowtail glycophorin which prepared by the waste from fish processing factory will be utilized as an antibiotic reagent. Furthermore, it is assumed that the possibb

ility of having antimicrobials is in not only carp, but also in the fish whole.

In teleost blood, IgG does not exist, and other antibodies exist in low levels. It is suggested that glycophorin may exist as a substitute for antibodies in teleost blood. Although the physiological function of human glycophorin has not yet been clarified, the structure of the human glycophorin O-linked tetra oligosaccharide is a simpler form than that of the carp's pentose. It is considered that IgG become a major component in the human immune system and that the bacteriostatic activity of human glycophorins has been lost in the process of evolution.

### References

1. Aoki T, Chimura K, Nobuhiro N, Yasuko M (2014) Isolation and characterization of glycophorin from carp red blood cell membranes. Membranes (Basel) 4(3): 491–508.

2. Aoki T, Chimura K, Hikaru S, Yasuko M (2014) Structure of a sialo oligosaccharide from glycophorin in carp red blood cell membranes. Membranes 4(4): 764-777.

3. Aoki T, Inoue T (2011) Glycophorin in red blood cell membranes of healthy and diseased carp, *Cyprinus carpio L*. J Fish Dis 34(7):573-576.