Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: January 31, 2024

Citation: Aoki T, 2024. Physiological Function of Oligosaccharides in Teleost Blood, Medical Research Archives, [online] 12(1). https://doi.org/10.18103/mra.v12i1.4999

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DOI
https://doi.org/10.18103/mra.v12i1.4999

ISSN: 2375-1924

RESEARCH ARTICLE

Physiological Function of Oligosaccharides in Teleost Blood

Takahiko Aoki

Laboratory of Quality in Marine Products, Graduate School of Bioresources, Mie University, 1577 Kurima Machiya-cho, Tsu, Mie 514-8507, Japan

Email: aoki@bio.mie-u.ac.jp

ABSTRACT

Oligosaccharides in human blood are involved in various blood group antigens. Sialo-oligosaccharide-rich glycoproteins (GPs) in red blood cell (RBC) membranes also carry M, N, Ss and other blood group antigens. However, teleost blood is not expressed as a blood group antigen. Our study showed that carp, yellow tail and red sea bream GPs exhibit broad-spectrum antibiotic activity. This bacteriostatic activity is caused by the sialo-oligosaccharides from GPs.

Based on NMR and GC–MS results, the structure of the bacteriostatic sialo-oligosaccharide from carp GP was determined to be NeuGcα2→6(Fucα1→4) (Glcα1→3) GalβI→4GalNAc-ol. Compared to carp pentose, the structure of human GP O-linked tetra- or tri-oligosaccharides is simpler. In addition, NeuAc is simpler in human GPs than in carp NeuGc.

In contrast to human blood, teleost blood does not contain IgG, and other antibodies occur at low levels. Teleost GPs may act as substitutes for antibodies, such as IgG, in the immune system. In this review, the transformation of the physiological function of oligosaccharides with respect to their structure is described.

Keywords: teleost, red blood cell membranes, glycophorin, antibiotic activity, blood group antigen, immune system

Abbreviations

GP: glycophorin;
RBC: red blood cell;
NeuGc: *N*-glycolylneuraminic acid;
Ac: acetyl;
Fuc: fucose;
Glc: glucose;
Gal: galactose;
GalNAc-ol: *N*-acetylgalactosaminitol;
NeuAc: *N*-acetylneuraminic acid;
CBB: Coomassie brilliant blue R-250

Introduction

The physiological function of oligosaccharides generated from glycoproteins or glycolipids in human blood involves the presence of various blood group antigens on the surface of human red blood cell (RBC) membranes[1]. In addition to sialo-oligosaccharide-rich glycoproteins in RBC membranes, glycophorins (GPs) carry M, N, Ss and other blood group antigens[2,3]. Immune reactions to blood group antigens are essential in transfusion. When the blood of other individuals is mixed, these blood group antigen polymorphisms lead to haemagglutination. However, when teleost blood from the same species is mixed, haemagglutinated does not occur. Based on this phenomenon, the blood group system in teleosts may be analogous to that in other individuals of the same species[4].

To clarify the physiological activity of teleost GPs, we isolated and prepared GPs from the RBC membranes of carp (*Cyprinus carpio* L.) according to the human GP preparation method[5]. Carp GP was found to exhibit bacteriostatic activity against various gram-negative and gram-positive bacteria, including fish pathogens[6]. This antibacterial property of carp results from the presence of the attached sialo-oligosaccharide (P-1)[6].

While MN and Ss blood group antigens are carried on human GPs, their antibacterial properties have not been detected. These differences in each GP were elucidated by the structure of the oligosaccharides in these GPs and blood group antigens.

Physiological activity of oligosaccharides as blood group antigens

Among the various blood group types, ABO blood group antigens are the most clinically important group in human blood transfusion practice. However, the role of oligosaccharides attached to these antigens has not been clearly determined for a long time because purifying and preparing oligosaccharides is difficult. Moreover, identification is difficult because oligosaccharides are attached to a diverse range of substrates.

Since 2000, the use of molecular biology, gene cloning, the sequencing of specific glycosyltransferase genes and other methods (NMR and X-ray crystallography) has led to studies on the physiological activity of oligosaccharides[7,8]. These results revealed that the ABO blood group antigen has three different terminal oligosaccharide sequences. These terminal saccharides present as much as 75% of the total antigens on N-linked glycans and reside on the major glycoprotein band 3 in RBC membranes[8]. Band 3 contains approximately 50% of all ABO antigens[8]. Furthermore, approximately 10% of ABO antigens reside on O-linked glycans, and approximately 15% reside on glycolipids in RBC membranes[8]. The terminal trisaccharide sequence of blood Group A is GalNAcα1→3 (Fucα1→2) Galβ1→. The sequence of blood Group B is Galβ1→ 3(Fucα1→2) Galβ1→. Blood Group O is the disaccharide Fucα1→2Galβ1→[8]. The main glycoprotein in human RBC membranes, GPs, carries M, N, Ss and other blood group antigens. These antigens rely on sialo-oligosaccharides, which attach to O-linked or N-linked oligosaccharides of GPs. Human GPs lose blood group activities after desialylation treatment [2].

In addition to GPs, several glycoproteins in human RBC membranes have blood group antigens[1], such as DARC/Duffy blood group antigens[9], Lu (Lutheran) blood antigen[10], and the Kell blood group antigen[11]. However, the physiological function of oligosaccharides against these antigens remains unclear[12].

Blood group antigens of teleost blood

As mentioned above, the diversity of blood group antigens in human RBCs is not observed in carp sera. In our experiments, haemagglutination was not observed when individual carp blood samples were mixed[13]. Many serological studies have examined the use of teleost serum against environmental antigens; however, this phenomenon has not been clearly observed in blood transfusions from the same species[14]. Suzuki reported that isohaemagglutinin in the serum of teleost blood is very rare and is expressed at low levels[4]. As different blood types have not been reported in carp, Takeda examined the possibility of blood transfusion from individual carp[15]. In these experiments, transfusion was effective in six patients in eleven trials. However, anaemia could not be effectively prevented in five patients.

Takeda concluded that blood incompatibility was possible between different individuals of carp[15]. In our study, MN and Ss blood group activities were not detected in carp blood by the haemagglutination titration test. However, the carp GPs exhibited weak N blood group activity, and a mixture of N and serum was observed under a microscope (Figure 1). These results suggested that the blood type was incompatible.

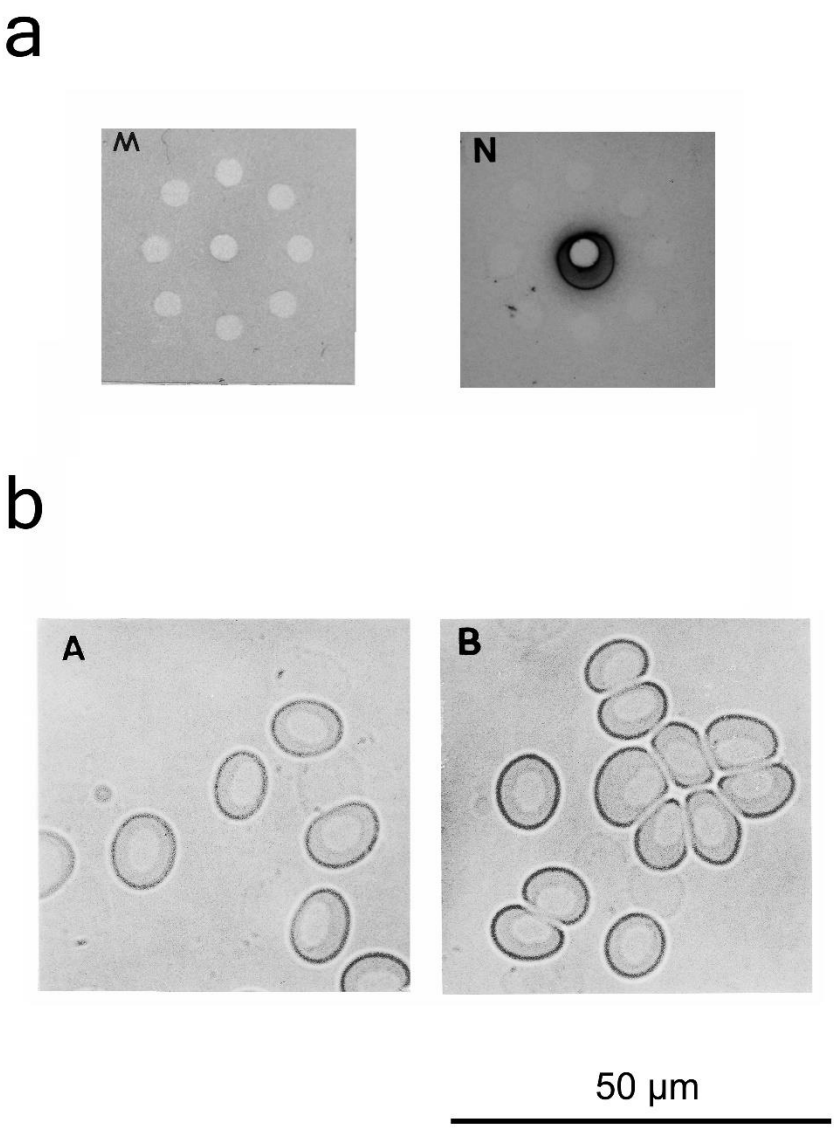

Figure 1. Immunological analysis of carp glycophorin.
(a) Double immunodiffusion analysis of carp glycophorin.
The immunodiffusion test was performed following the methods of Ouchterlony[30]. The centre wells contained M, anti-M serum (rabbit); N, anti-N serum (rabbit). The outer wells contained the carp GP solution, starting at twelve o'clock, after which the dilutions were doubled clockwise. The diameter of the well was 2 mm. After incubation was performed at 37 °C for 48 h, the precipitate was identified by staining the precipitate with 5% CBB.
(b) Haemagglutination of carp red blood cells with anti-N serum.
A, Packed carp RBCs were washed with fish saline (1.7% NaCl in 1 mM Tris, pH 8.0)[31] and suspended in the same saline (10%, v/v); B, An equal volume of anti-N serum was added to the cell culture mixture at 25 °C.

Physiological activity of sialo-oligosaccharides from teleost GPs

To clarify the physiological activity of carp GPs, we performed a sensitivity test for the growth of several bacteria using the disk plate method. These results suggested that the carp GPs exhibited bacteriostatic activity, and this activity was observed for 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*)[16,17]. Using a sensitivity test, sialo-oligosaccharides from the P-1 fraction were revealed to exhibit bacteriostatic activity; in contrast, this activity was not observed for the glycolipid fraction from carp RBC membranes and the human GP fraction[16].

Using the asialo P-1 fraction for NMR analysis and the permethylated P-1 fraction for GC–MS, we determined that the structure of the bacteriostatic P-1 strain was NeuGcα2→6(Fucα1→4)(Glcα1→3)Galβ1→4GalNAc-ol[18].

Antimicrobial activity was observed on the carp GP as well as the yellow tail and red sea bream GPs[19]. The carp are freshwater fish, and the yellow tail and red sea bream are marine red-flesh fish and white-flesh fish, respectively. Thus, we concluded that this antimicrobial activity occurs not only in these teleost species but also in all fish. Teleost GPs may exist as substitutes for antibodies such as IgG in teleost blood within the immune system.

Physiological role of Igs in teleost blood

In human blood, immunological reactions, including antimicrobial activity, are performed by immunoglobulins (Igs). IgG does not exist in teleost blood, and IgM and IgD exist only in teleost blood[20,21]. However, these antibodies occur at relatively low levels compared to those in human blood[22,23]. Unlike other groups of vertebrae, IgM is the main class of immunoglobulin in most teleost fishes[24]. IgM is tetrameric in teleostei and pentameric in elasmobranchs[25]. Most carp IgMs have tetrameric structures with a molecular weight of approximately 760 kDa[20]. Compared to monomeric IgG, IgM may not easily permeate vessel walls due to its complexity and large size. Therefore, IgM is distributed not only in the blood but also in the head kidney and spleen, which contain IgM-producing cells in mandarin fish, *Siniperca chuatsi* [26].

On the other hand, IgD was discovered in teleost blood in 1997[21], and its function became clear in 2018[27]. Moreover, teleost IgM and IgD have been reported to elicit mammalian-like mucosal immune responses[28]. The role of these Igs may be attributed to the mucosal epithelial barrier against potential pathogens.

As the antimicrobial activity of human GPs has not been reported and various Igs in human blood have shown immunological efficacy, teleost GPs may act as a substitute for antibodies in blood.

Conclusion

The most commonly studied human GP oligosaccharides include the following trisaccharides: Gaβ1→3(NeuAcα2→6)GalNAc-ol and NeuAcα2→3Galβ1→3GalNAc-ol. However, a NeuGc-containing O-linked oligosaccharide has also been reported from horse, pig, goat and rabbit GPs, and the most reported structure is the following trisaccharide: Galβ1→3(NeuGcα2→6) GalNAc-ol[12]. The core structure of GPs may be a trisaccharide.

Interestingly, the core structures of the terminal sequences of blood Groups A and B are trisaccharides, GalNAcα1→3(Fucα1→2) Galβ1→ and Galβ1→3(Fucα1→2) Galβ1→, respectively[8]. While the structure of the antimicrobial oligosaccharide from carp GPs was a sialopentasaccharide, the monosaccharides used were similar to those used for ABO blood group antigens or human GPs. Considering the core structure of physiological activities as a trisaccharide, Glc and Fuc are cleaved from sialo-pentasaccharide, and its structure leads to the trimer of human GP (Figure 2). Because teleost GPs or human GP A and B proteins do not connect to the cytoskeleton[12,29], attached sialic acid contributes to the topology of GPs in RBC membranes. In contrast to human GP sialo-oligosaccharides, O-linked triosaccharides are simpler than carp pentoses. Human NeuAc is also simpler than carp NeuGc. The antibacterial property of human GPs may have been lost during evolution[12].

As Glc and NeuGc are released from sialopentasaccharides, they combine to form the trimer of the A blood antigen (Figure 2). Various glycosyltransferases are encoded within the genome and are widely expressed; moreover, these enzymes are not strictly specific for substrates[8]. The sialo-oligosaccharides of GPs may be transformed to ABO antigens. While GP loses the antibacterial property of sialo-oligosaccharides, IgG presumably emerges from IgM over the process of evolution. According to

Magnadóttir, all immunoglobulin classes are glycosylated, and compared with IgG, IgM is characterized by a relatively high carbohydrate content[22]. The contained oligosaccharide may be cleaved by glycosidases, leading to the formation of IgG from IgM. IgG is a major component of the human immune system, and the bacteriostatic activity of human GPs has been lost during evolution.

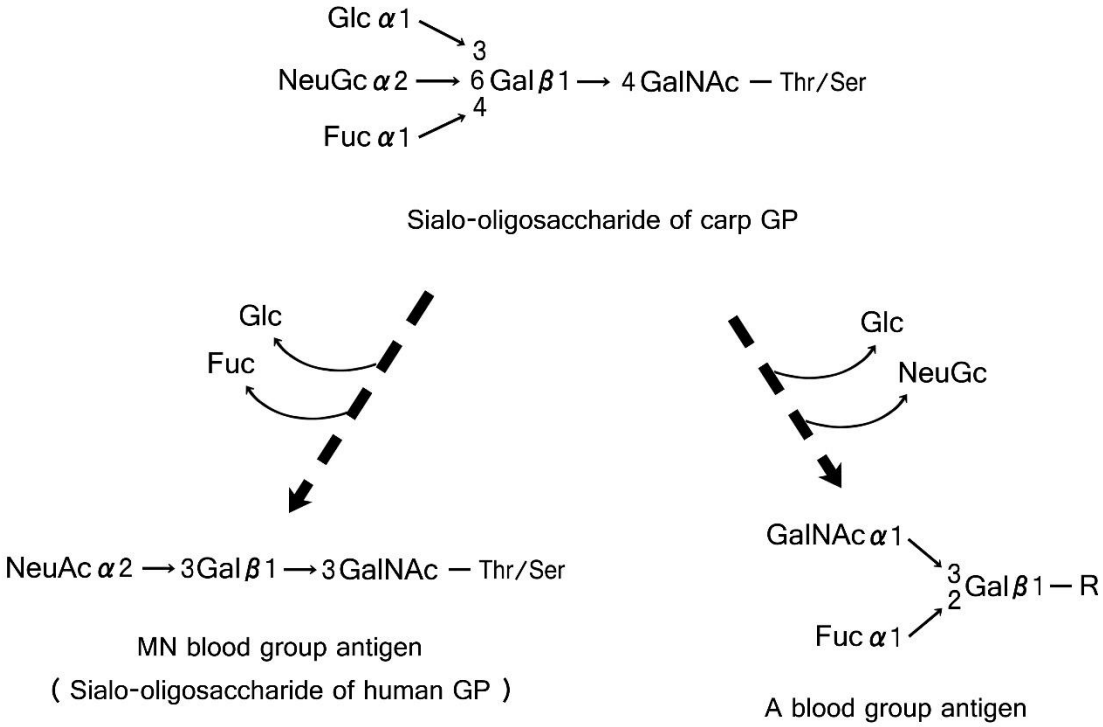

Figure 2. Terminal saccharides of blood group antigens and sialo-oligosaccharides of carp GPs. R: N-glycan, O-glycan or lipid.

References

1. Anstee DJ. Blood group-active surface molecules of the human red blood cell. *Vox Sang*. 1990;58:1–20.
2. Prohaska R, Koerner TAW Jr., Armitage IM, Furthmayr H. Chemical and carbon-13 nuclear magnetic resonance studies of the blood group M and N active sialoglycopeptides from human glycophorin A. *J. Biol. Chem*. 1981;256:5781–5791.
3. Dahr W, Beyreuther K, Steinbach H, Gielen W, Krüger J. Structure of the Ss blood group antigens, II. A methionine/threonine polymorphism within the N-terminal sequence of the Ss glycoprotein. *Hoppe-Seyler Z. Physiol. Chem*. 1980;361:895–906.
4. Suzuki A. Blood type of fish. *Bull. Jpn. Soc. Sci. Fish*. 1967;33:372-381.
5. Marchesi VT, Andrews EP. Glycoproteins: Isolation from cell membranes with lithium diiodosalicylate. *Science*. 1971;174:1247-1248.
6. Aoki T, Chimura K, Nakao N, Mizuno Y. Isolation and characterization of glycophorin from carp red blood cell membranes. *Membranes*. 2014;4:491-508.
7. Morgan WTJ, Watkins WM. Unravelling the biochemical basis of blood group ABO and Lewis antigenic specificity. *Glycoconj. J*. 2000;17:501-530.
8. Misevic G. ABO blood group system. *Asia Pacific J. Blood Types Genes* 2018;2:71-84.
9. Grodecka M, Bertrand O, Karolak E, Lisowski M, Waśniowska K. One-step immunopurification and lectinochemical characterization of the Duffy atypical chemokine receptor from human erythrocytes. *Glycoconj. J*. 2012;29:93–105.
10. Daniels G, Khalid G. Identification, by immunoblotting, of the structures carrying Lutheran and para-Lutheran blood group antigens. *Vox Sang*. 1989;57:137–141.
11. Jaber A, Blanchard D, Goossens D, Bloy C, Lambin P, Rouger P, Salmon C, Cartron J-P. Characterization of the blood group Kell (K1) antigen with a human monoclonal antibody. Blood. 1989;73:1597–1602.
12. Aoki T. A comprehensive review of our current understanding of red blood cell (RBC) glycoproteins. *Membranes*. 2017;7:56-74
13. Aoki T. Preparation and structural analysis of a sialo-oligosaccharide from glycophorin in carp red blood cell (RBC) membranes. *Med. Res. Arch*. 2023;11. doi:10.18103/mra.v11i3.3644.
14. Alexander JB, Ingram GA. Noncellular nonspecific defence mechanisms of Fish. *Annual Rev. of Fish Diseases*. 1992;2:249-279.
15. Takeda T. Effects of Blood sampling on the hematocrit value in the carp and trials of blood transfusion for prevention of anemia. *Sci. Bull. Fac. Agr., Kyushu Univ*. 1985;39:153-157.
16. Aoki T, Chimura K, Nakao N, Mizuno Y. Isolation and characterization of glycophorin from carp red blood cell membranes. *Membranes*. 2014;4:491-508.
17. Aoki T, Inoue T. Glycophorin in red blood cell membranes of healthy and
diseased carp, *Cyprinus carpio* L. *Journal of Fish Diseases*. 2011;34:573-576.
18. Aoki T, Chimura K, Sugiura H, Mizuno Y. Structure of a sialo-oligosaccharide from glycophorin in carp red blood cell membranes. *Membranes*. 2014;4:764-777.
19. Aoki T. Determination of the bacteriostatic activity of glycophorin preparations from red blood cell (RBC) membranes of yellow tail and red sea bream. *Medical Research Archives*. 2021;9. doi: 10.18103/mra.v9i12.2613.
20. Bag MR, Rajendran MKV, Mukherjee SC. Characterization of IgM of Indian major carps and ther cross-reactivity with anti-fish IgM antibodies. *Fish Shellfish Immunol*. 2009;26:275-278.
21. Wilson M, Bengtén E, Miller NW, Clem LW, Du Pasquier L, Warr GW. A novel chimeric Ig heavy chain from a teleost fish shares similarities to IgD. *Proc. Natl. Acad. Sci. USA*. 1997;94:4593-4597.
22. Magnadóttir B. Comparison of immunoglobulin (IgM) from four fish species. *ICEL. AGR. SCI*. 1998;12:47-59.
23. Solem ST, Stenvik J. Antibody repertoire development in teleosts-a review with emphasis on salmonids and *Gadus morhua* L. Dev. Comp. Immunol. 2006;30:57-76.
24. Mochida K, Lou Y-H, Hara A, Yamauch K. Physical biochemical properties of IgM from a teleost fish. *Immunology* 1994;83:675-680.
25. Voss EW, Sigel MM. Valence and temporal change in affinity of purified 7S and 18S nurse shark anti-2,4-dinitrophenyl antibodies. *J. Immunol*. 1972;109:665-673.
26. Tian J, Sun B, Luo Y , Zhang Y, Nie P. Distribution of IgM, IgD and IgZ in mandarin fish, *Siniperca chuatsi* lymphoid tissues and their transcriptional changes after *Flavobacterium columnare* stimulation. *Aquaculture*. 2009;288:14-21.
27. Gutzeit C, Chen K, Cerutti A. The enigmatic function of IgD: some answers at last. *Eur. J. Immunol*. 2018;48:1101-1113.
28. Yu Y, Wang Q, Huang Z, Ding L, Xu Z. Immunoglobulins, Mucosal immunity and vaccination in teleost fish. *Frontiers in Immunology* 2020;doi:10.3389/fimmu.2020.567941.
29. Aoki T. Behaviour of a sialo-oligosaccharide from glycophorin in teleost red blood cell membranes. In Animal models and experimental

research in medicine, 2022; doi:10.5772/intechopen.107234.
30. Ouchterlony Ö. Antigen-antibody reactions in gels: IV. types of reactions in coordinated systems of diffusion. *Acta Path. Micr. Scand.* 1953;32:231-240.
31. Riggs A. Preparation of blood hemoglobins of vertebrates. In *Methods Enzymol. – 76* (Antonini E, Rossi-Bernardi L & Chiancone E eds), 1981 (pp. 5-29). Academic Press, USA.