RESEARCH ARTICLE

Determination of the Bacteriostatic Activity of Glycophorin Preparations from Red Blood Cell (RBC) Membranes of Yellow Tail and Red Sea Bream

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Abstract

Glycophorins (GPs) in red blood cell (RBC) membranes of carp (*Cyprinus carpio* L.) exhibit bacteriostatic activity against various gram-negative and gram-positive bacteria. To clarify the physiological activity of teleost fish GPs other than those from carp, we performed a sensitivity test using GP preparations from the RBC membranes of yellow tail and red sea bream. The sensitivity test for the growth of *Micrococcus luteus* was performed using the disc-plate method. These results suggested that the yellow tail and red sea bream GPs exhibit antibiotic activities.

Keywords: teleost, yellow tail, red sea bream, red blood cell membranes, glycophorin, antibiotic activity.

Introduction

Glycophorins (GPs) are transmembrane glycoproteins that contain sialic acid. These glycoproteins are found in the red blood cell (RBC) membranes of humans [1-4] and other mammals [5-8] and birds [9, 10]. We examined the GPs in the RBCs of teleosts [11], and demonstrated that teleost GPs isolated from RBC membranes of carp (*Cyprinus carpio* L.), inhibit bacterial growth by attachment to the flagellum of *Vibrio anguillarum* or the cell surface of *Micrococcus luteus* [12]. These antibacterial activities are caused by sialo-oligosaccharides from carp GPs and are attributed to the nature of the lectin receptor [13]. 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 indicate that carp GPs are released from RBC membranes and adsorbed onto the surface of invading bacteria in the blood [14].

Moreover, the adsorbent properties of carp GPs appear to be a broad-spectrum antibiotic, showing reactions to all other test bacteria (*Bacillus subtilis*, *Edwardsiella tarda*, *Aeromonas hydrophila*, *Escherichia coli* and *Pseudomonas fluorescens*) [12, 15]. These antibacterial activities have not been reported for human or other mammalian GPs [14]. While human GP has been reported to carry several blood group antigens [16-20], its physiological activity is unclear [14].

Our objective was to clarify the physiological activity of GPs from fish other than carp, and we performed a sensitivity test using GP preparations from RBC membranes of yellow tail and red sea bream.

Experimental methods

Yellow tail (*Seriola quinqueradiata*) (body weight: 6.0 kg) was obtained from Mie Gyoren, and red sea bream (*Pagrus major*) (mean body weight: 1.4 kg) was obtained from Owase Bussan Co.,Ltd, Owase, Japan**.** Ficoll-Paque PLUS was purchased from Amersham Biosciences (GE Healthcare, Little Chalfont, U.K.). The *M. luteus* ATCC 9341 strain was cultured in our laboratory. The micro-organic media were purchased from Nissui Pharmaceutical Co. (Tokyo, Japan). All other reagents were analytical grade.

Blood was collected by cutting the dorsal aorta at an upper base of the gill lid using a knife and holding the fish in a vertical position. The blood was diluted 1:1 with fish Ringer (145 mM NaCl, 5 mM $CaCl_2$, 1 mM $MgSO_4$, 4 mM KCl,

10 mM HEPES, and 5 mM glucose; pH 7.9) [21]. In the case of clot formation in the blood, the diluted blood was then homogenized with a tight-fitting Dounce homogenizer (10 strokes). After dilution, the white blood cells and plasma were removed from the blood using Ficoll-Paque PLUS. The RBC membranes were prepared as described previously [12]. The GP from each RBC membrane preparation was extracted as described previously using lithium 3,5-diiodosalicylate (LIS)-phenol [12]. These GP preparations were stored at -80ºC until use.

Protein content was determined by the method of Lowry *et al.* [22] with bovine serum albumin as the standard. The sensitivity test for the growth of *M. luteus* was performed using the disc-plate method. The agar medium containing bacteria (10^{6-8} cfu/10 mL medium) was layered on each plate medium and dried for 30 min. A paper disk (8 mm diameter, Advantec Toyo Kaisha, Ltd., Tokyo, Japan) containing each fraction was placed on the medium and incubated at 20°C. After 24 h, the inhibition zone was observed on each plate. The composition of the plate medium was 2.5% heart infusion broth"*Nissui*" containing 1.5% agar. PBS (phosphate-buffered saline) was used as a control.

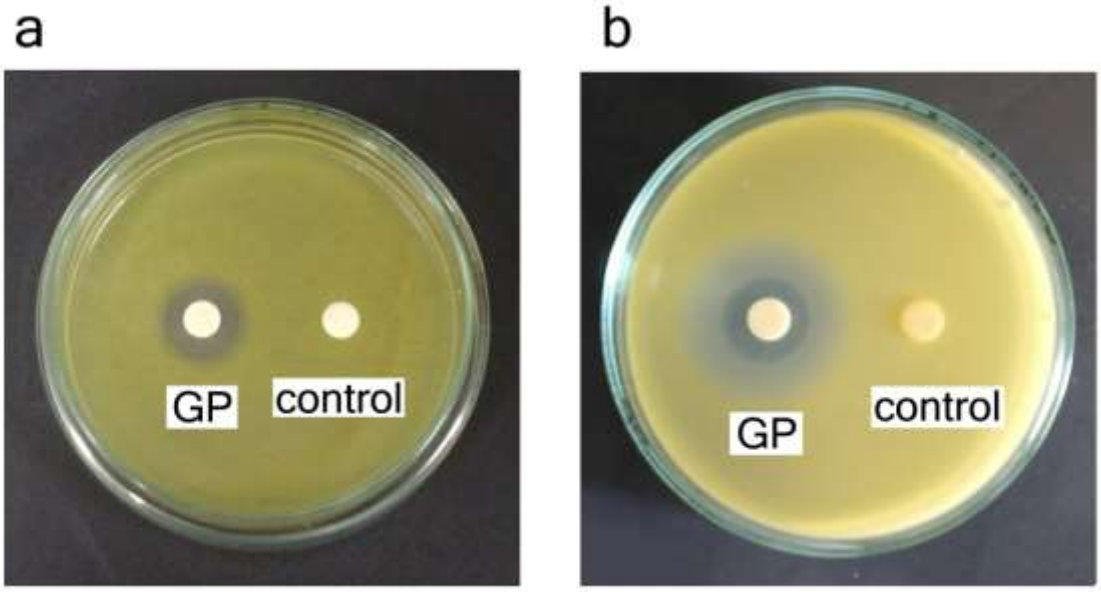

Figure 1. Sensitivity test for *M. luteus* by the disc-plate method.
(a) Glycophorin preparation from yellow tail (ca. 10 μg protein/disc). GP, yellow tail GP; control, PBS; (b) Glycophorin preparation from red sea bream (ca. 10 μg protein/disc). GP, red sea bream GP; control, PBS

Results and Discussion

M. luteus formed inhibition zones around the paper discs containing the yellow tail GP or red sea bream GP fraction (Figure 1). While the outer zone of *M. luteus* did not produce yellow pigments, the inner zone of *M. luteus* did not produce pigments. In contrast, the inhibition zone was not formed around the paper discs containing PBS.

These results showed that not only carp GP preparations but also the yellow tail and red sea bream GPs had antibiotic activities. In our previous study, we performed a sensitivity test using carp GPs for the growth of *M. luteus* [12]. Double inhibition zones around the paper disc containing the carp GPs in this test were suggested that the adsorbent properties of yellow tail GPs and red sea bream GPs appear to be the same as that of carp GPs.

These results suggested that the yellow tail or red sea bream GPs have a broad-spectrum antibiotic activity. While carp are freshwater fish, yellow tail and red sea bream are marine red-flesh fish and white-flesh fish, respectively. Thus, we concluded that this antimicrobial activity is not only confined to these teleost species but can be found all fish.

In teleost blood, IgG does not exist, and other antibodies exist at low levels [23]. GP may be a substitute for antibodies in teleost blood. IgG is considered a major component in the human immune system and the antibiotic activity of human GPs has been lost in the process of evolution.

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