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Characterization of the Classical Complement Pathway of Rainbow Trout

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Characteristics of the classical complement pathway (CCP) of rainbow trout *Salmo gairdneri* was examined using sheep red blood cells sensitized with porgy *Pagrus major* antibody which is compatible with rainbow trout complement. The rainbow trout CCP showed the highest activity when the reaction was performed at 20-25°C and around pH 7.5, and the hemolysis reached a plateau in about 120 min. It was found that a considerable hemolysis which is attributable to the alternative complement pathway (ACP) of rainbow trout took place in the presence of 10 mM Mg^{2+}. The hemolysis caused by the ACP, however, was less than 10% of the total hemolysis when the hemolytic reaction was performed in the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+}.

INTRODUCTION

In previous studies (Yano et ***al., 1984*** ; Matsuyama et *al*., 1985 ; Yano et ***al.,*** 1988), ***we*** investigated the optimum conditions for the assay of the classical complement pathway (CCP) activity of carp *Cyprinus carpio*, porgy *Pagrus* ***major*** and tilapia *Tilapia nilotica* by use of sheep red blood cells (SRBC) sensitized with the antibodies of these fishes.

In the present work, we examined the optimum conditions for the assay of the CCP activity of rainbow trout *Salmo gairdneri* using SRBC sensitized with porgy antibody which is compatible with rainbow trout complement.

MATERIALS AND METHODS

Reagents

The sources of reagents were as follows : EDTA (ethylenediaminetetraacetic acid) and barbital sodium, Wako Pure Chemical Industries Co., Osaka ; Amberlite IRC-50, Organo Co., Tokyo ; sheep blood in Alsever's solution, Japan Bio-Supply Center, Tokyo.

The buffers used in the experiment and their abbreviations were as follows : GVB^{2+}, veronal-buffered saline (pH 7.4) containing 0.1% gelatin, 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$; EDTA•GVB, veronal-buffered saline (pH 7.4) containing 0.1% gelatin and 10 mM EDTA ; GVB(f), veronal-buffered saline (pH 7.4), free of divalent cations, containing 0.1% gelatin. GVB(f) was made by passing 200 ml of veronal-buffered saline containing 0.1% gelatin through an Amberlite IRC-50 column (5 ml) equilibrated with 0.15 M NaCl.

Rainbow trout serum

Blood was collected from the caudal vessel of rainbow trout, weighing about 250 g, clotted at room temperature for 30 min and then cooled at 0°C for 1 h. After centrifugation, the serum was frozen in liquid nitrogen and stored at -80°C.

Preparation of sensitized sheep red blood cells (EA)

EA (sheep red blood cells sensitized with porgy antibody) were prepared as described in the previous paper (Matsuyama *et al.,* 1985) and suspended in GVB^{2+} at a concentration of 5×10^8 cells/ml. This EA suspension was stored at 4°C and used within one week.

Removal of divalent cations from rainbow trout serum

Rainbow trout serum (0.2 ml) was applied to an Amberlite IRC-50 column (3 ml) equilibrated with GVB(f) and eluted with the same buffer. The first eluate (0.5 ml) was discarded, and the next eluate (4 ml) was used as serum(f).

RESULTS

Effect of incubation temperature on the activity of the rainbow trout CCP

The reaction mixture composed of 0.10 ml of EA suspension (5×10^8 cells/ml), 0.25 ml of diluted rainbow trout serum (1/60) and 0.40 ml of GVB^{2+} was incubated at various temperatures ranging from 0 to 37°C for 30 min. The reaction was stopped by adding 0.75 ml of EDTA•GVB and, after centrifugation, percent hemolysis was calculated from the absorbance at 541 nm of the supernatant. Fig. 1 shows that rainbow trout complement was most active at 20–25°C.

Effect of pH on the activity of the rainbow trout CCP

The pH of GVB^{2+} was readjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.5 with 1 N HCl or 1N NaOH, and the hemolytic reactions were carried out at 20°C for 40 min. After

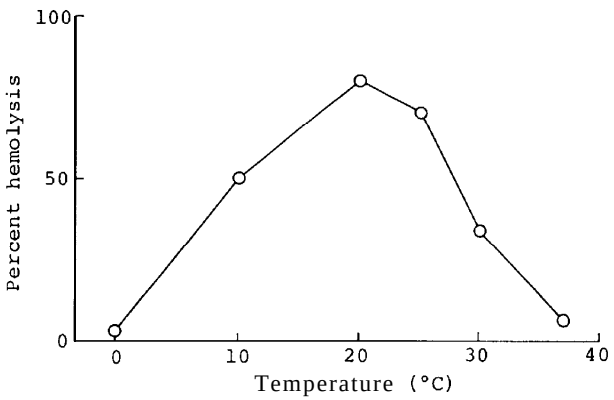

Fig. 1. Effect of incubation temperature on the classical complement pathway activity of rainbow trout. The hemolytic reaction was performed at 0 to 37°C for 30 min at pH 7.4.

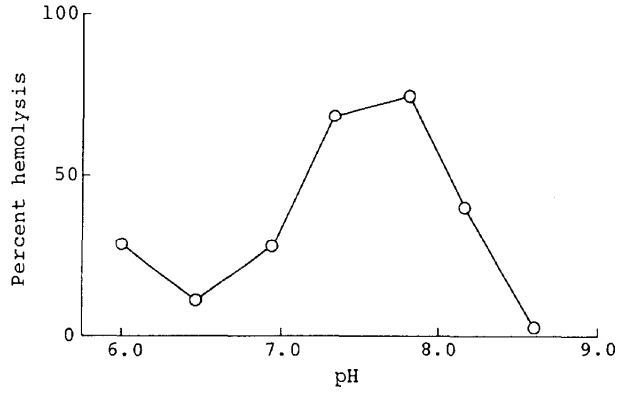

Fig. 2. Effect of pH on the classical complement pathway activity of rainbow trout. The hemolytic reaction was performed at 20°C for 40 min.

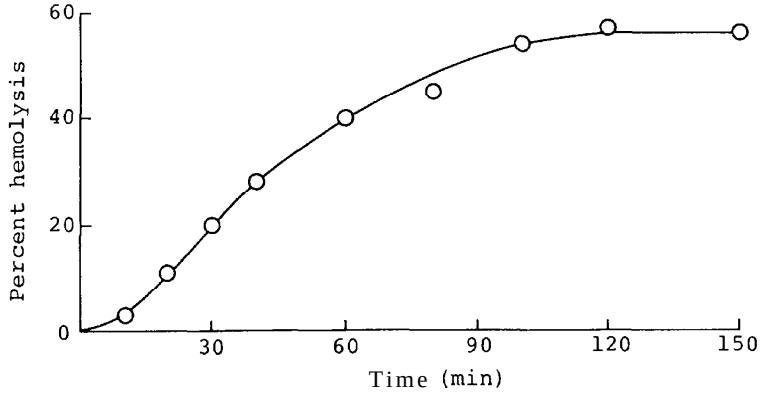

Fig. 3. Time course of hemolysis in the reaction of rainbow trout complement with sensitized SRBC. The hemolytic reaction was performed at 20°C for 150 min at pH 7.5.

addition of 0.75 ml of EDTA•GVB, each mixture was centrifuged and the percent hemolysis was calculated. As shown in Fig. 2, the highest activity was observed around pH 7.5.

Time course of hemolysis

The reaction mixture (7.5 ml) was incubated at 20°C for 150 min at pH 7.5. At timed intervals, an aliquot (0.75 ml) of the reaction mixture was withdrawn and mixed with 0.75 ml of EDTA•GVB, and percent hemolysis at each time point was calculated (Fig. 3). Hemolysis took place after a lag-time of about 10 min and, proceeding rather slowly, reached a plateau in about 120 min.

Effect of concentrations of Ca^{2+} and Mg^{2+} on the activity of the rainbow trout CCP

To a reaction mixture composed of 0.25 ml of serum(f), 0.10 ml of EA suspension

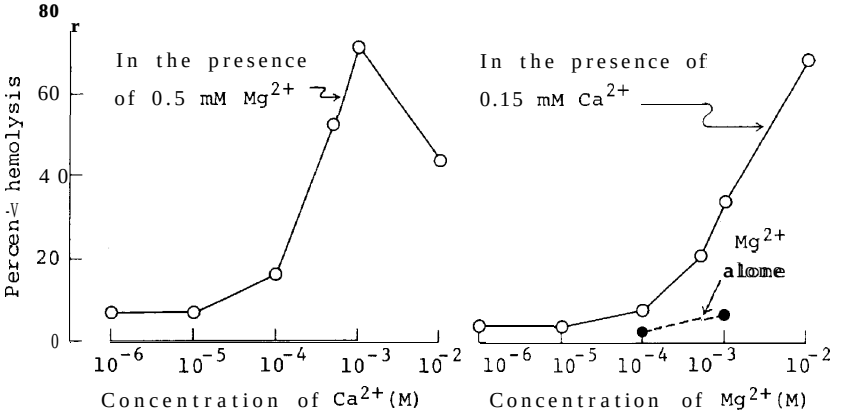

Fig. 4. Effect of concentrations of Ca^{2+} and Mg^{2+} on the classical complement pathway activity of rainbow trout. The hemolytic reaction was performed at 20°C for 40 min.

(5 x 10^8 cells/ml GVB (f)) and 0.40 ml of GVB (f) were added 5 μl of $CaCl_2$ solution and 5 μl of $MgCl_2$ solution. The mixture was incubated at 20°C for 40 min at pH 7.5. The concentrations of Ca^{2+} was altered from 10^{-6} to 10^{-2} M in the presence of 0.5 mM Mg^{2+}, and the concentration of Mg^{2+} was altered from 10^{-6} to 10^{-2} M in the presence of 0.15 mM Ca^{2+}. As a result, the highest activity was observed at 10^{-3} M Ca^{2+} and at 10^{-2} M Mg^{2+} (Fig. 4). Slight hemolysis was observed when the reaction mixture was incubated in the presence of 10^{-4} and 10^{-3} M Mg^{2+}, but absent of Ca^{2+}. This indicates that the alternative complement pathway of rainbow trout was activated to some extent by SRBC under these conditions.

DISCUSSION

Although the assay of the CCP activity of mammals is carried out using SRBC sensitized with rabbit antibody, the complement of fish is incompatible with rabbit antibody. Therefore, the assay of the CCP activity of fishes has been performed using SRBC sensitized with the antibody of homologous fish (Yano et al., 1984 ; Matsuyama et al., 1985 ; Yano et ***al.,*** 1988) or that of heterologous fish which is compatible with the complement of the test fish (Legler and Evans, 1967 ; Legler ***et al.,*** 1967). In this experiment, we examined the properties of the CCP of rainbow trout utilizing SRBC sensitized with porgy antibody which is compatible with rainbow trout complement.

The CCP of rainbow trout showed highest activity under following conditions: reaction temperature, 20-25°C ; pH 7.5 ; concentrations of Ca^{2+} and Mg^{2+}, 1 mM and 10 mM, respectively.

In a preliminary study, we observed that rainbow trout serum hemolyzed SRBC to a considerable extent in the presence of 10 mM Mg^{2+}. This hemolysis is attributable to the alternative complement pathway of rainbow trout. However, the hemolysis by the ACP was less than 10% of the total hemol'ysis when the hemolytic reaction was performed in the presence of 1 mM Ca^{2+} and 1 mM Mg^{2+}. This indicates that the CCP

activity of rainbow trout can be assayed under these conditions.

We have shown in the previous paper (Yano et al., 1988) that the titration of the CCP activity (CH50) of carp, porgy and tilapia can be performed under an uniform conditions as follows : reaction temperature, 25°C ; pH, 7.5 ; concentrations of Ca^{2+} and Mg^{2+}, 1.0 mM and 1.0 mM, respectively. The present experiment showed that the activity of rainbow trout CCP could also be assayed under the same conditions.

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REFERENCES

Legler, D. W. and E. E. Evans 1967 Comparative immunology : Hemolytic complement in elasmobranchs. *Proc. Soc. Exp. Biol. Med.*, **124** : 30-34

Legler, D. W., E. E. Evans and H. K. Dupree 1967 Comparative immunology : Serum complement of fresh water fishes. *Trans. Am. Fish. Soc., 96: 237-242*

Matsuyama, H., A. Hirata, M. Nakao and T. Yano 1985 Optimum conditions for the assay of hemolytic complement titer of porgy (*Pagrus major)* serum. J. *Fac. Agr., Kyushu Univ., 30 :* 149-*158*

Yano, T., H. Ando and M. Nakao 1984 Optimum conditions for the assay of hemolytic complement titer of carp and seasonal variation of the titers. *J. Fac. Agr., Kyushu Univ., 29:* 91-101

Yano, T., Y. Hatayama, H. Matsuyama and M. Nakao 1988 Optimum conditions for the assay of the classical pathway-complement titer of tilapia (*Tilapia nilotica)* serum. *J. Fac. Agr., Kyushu Univ., 33: 29-36*