Preparation of Antiserum against Carp (Cyprinus carpio L.) Immunoglobulin and Its Application for Immunohistochemistry

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In the cultivation of teleost fish, immunological protection against infectious diseases is an important problem. It is essential to clarify the fundamental differentiation of the cell system for immunoglobulin production in the teleost fish as has been done in many species of mammals [2, 4, 6, 8, 10, 12]. For studies of immunoglobulin-producing cells and related cells in the teleost, a monospecific antibody against teleost immunoglobulin is necessary. In this paper a procedure for the preparation of a monospecific antibody against carp immunoglobulin is described and its availability for immunohistochemical studies is discussed.

Preparation of carp immunoglobulin: Carp were immunized with bovine serum albumin (BSA). One milliliter of saline solution containing 20 mg of BSA was mixed with 1 ml of Freund’s complete adjuvant (Yatoron Co., Tokyo, Japan), and 2 ml of the mixture was intraperitoneally injected into 2 carp (weighing 650 g, kept in 23°C filtered water). After 2 months, a booster injection with the mixture was given. The carp were bled by cardiac puncture 14 or 21 days after the booster injection, and the serum was separated. Immunoglobulin was salted out with ammonium sulfate at 50% saturation. The precipitate was collected by centrifugation at 10,000 × g for 30 min, and was dissolved in a minimum volume of 0.1 M phosphate buffer solution containing 0.15 M NaCl, pH 7.4, (PBS). The solution was gel filtered through a column (2.5 × 85 cm) of Sephadex G-200 (Pharmacia, Uppsala, Sweden) with PBS at a flow rate of 8 ml/hr at 4°C. The first peak was dialyzed against 0.1 M Tris-HCl buffer, pH 8.0, and was applied on a column (1 × 12 cm) of DEAE cellulose (DE23, Whatman Biochemicals Ltd., U.K.) equilibrated with the same buffer. Elution was performed by the linear-gradient method with 0 to 0.2 M NaCl in Tris-HCl buffer, pH 8.0. Figure 1a shows the elution profile obtained by DEAE cellulose chromatography. Anti-BSA activity was detected in the fractions of the first peak, which were eluted with about 0.1 M NaCl. Figure 1b shows a single protein band that was detected at same position as human α1-macroglobulin (molecular weight 700,000 ~ 750,000) by electrophoretic analysis [11].

Preparation of specific antiserum against carp immunoglobulin: A 0.7 ml portion of the peak fraction of the DEAE cellulose chromatography containing 500 μg of protein was mixed with 0.7 ml of Freund’s complete adjuvant and the mixture was intradermally injected into a rabbit, followed by an intravenous booster injection with the same volume of the fraction 14 days after the first injection. The rabbit was bled 7 days after the booster injection, and the serum was separated. The crossreactive antibodies against non-immunoglobulin materials in the serum were absorbed with normal carp hepatocytes. Figure 2 shows a single precipitin line that was formed in immuno-electrophoresis [7], indicating that the serum did not contain any crossreactive antibodies against other materials. This preparation is called anti-carp immunoglobulin serum (anti-Clg) in this paper.

Application of antiserum to immunohistochemistry: Head kidneys were removed from normal or immunized carp with BSA. For light microscopical analysis, the tissues were fixed in cold periodate-lysine-paraformaldehyde solution for 24 hr. Four micrometer paraffin sections were prepared and immunostained by the peroxidase anti-peroxidase method with anti-Clg. Figure 3a shows a strongly immunoreactive plasmacytoid cell. For immunoelectron microscopic analysis, the tissues were fixed in a solution of 2% paraformaldehyde and 2% glutaraldehyde for 2 hr at 4°C, and were embedded in Lowicryl K4M (Chemische Werke Lowi, Germany). Ultrathin sections were incubated with anti-Clg, and then with protein A-gold complex (15 nm, E-Y Lab., Inc., U.S.A.), followed by staining with aqueous.
Fig. 1. (a) Ion-exchange chromatography of carp serum Ig on a column of DE23 DEAE cellulose (line). A large peak is detected at an NaCl concentration of less than 0.1 M. The peak fractions contain BSA antibody activity (arrow heads). Protein was monitored by absorbance at 280 nm. Each fraction contained 2.5 ml. The dotted line shows the NaCl concentration. (b) Electrophoretical analysis of fraction No. 14 of (a) and carp whole serum (WS). Fraction No. 14 contains an electrophoretical homogeneous protein.

Fig. 2. Immunelectrophoretical analysis of rabbit antiserum against carp Ig (anti-CIg). Anti-CIg precipitates in a single line with carp normal serum (NS), and immunized carp serum (IS) which contains BSA antibody activity. uranyl acetate and lead citrate. Gold particles were found in the cisternae of the rough endoplasmic reticulum of plasmacytoid cell (Fig. 3b). These results indicate that this anti-CIg is available for light and electron microscopical immunoanalysis of the carp immunoglobulin-producing cells.

It has been reported that teleost fish have only one type of immunoglobulin, tetrameric IgM-like immunoglobulin [1, 3, 5, 9, 13]. The chromatographical elution characteristics of carp immunoglobulin in this study were in good agreement with the case of trout immunoglobulin [3], and the obtained protein was an electrophoretically homogeneous macroglobulin. Moreover, it is suggested that anti-CIg is available to study the distribution and differentiation of immunoglobulin-producing cells in carp lymphoid tissue. These procedures for the purification of carp immunoglobulin and for the preparation of specific anti-CIg described in this paper may be applicable to the purification of immunoglobulin and to the preparation of anti-immunoglobulins of other types of fish.

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Fig. 3. Light- and electron-microscopic immunohistochemical analyses of immunized carp head kidney. The plasmacytoid cell is strongly positive in the parenchyma of the head kidney (a). Immunoelectron microscopically, a number of gold particles (15 nm) are found in the dilated cisternae of the rough endoplasmic reticulum (b). a: ×1,750, b: ×21,000.

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REFERENCES

要約

コイ免疫グロブリンに対する抗血清の作製とその免疫組織化学への応用（短報）：今川智敏・橋本善春・首藤文業1）・畠 泰寛・杉村 誠（北海道大学獣医学部家畜解剖学教室，1）家畜生化学教室）——コイ血清から穂安塩析、ゲルろ過、イオン交換クロマトグラフィーにより免疫グロブリンを精製した。それに対するウサギ抗血清を得、コイ肝細胞で吸収することにより特異的な抗血清を作製した。得られた血清により顕眼内形質細胞が強く染色され、本血清はコイの抗体産生細胞の光顕および電顕免疫組織化学的検索に応用可能であると考えられた。