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# Structural Characterization of an IgM-like Immunoglobulin in the Serum of Swamp Eel, *Monopterus albus*

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**IgM-like immunoglobulin was purified from the immune serum of *M. albus* which immunized with bovine serum albumin(BSA) as an antigen(Ag) and characterized. The Ag-specific antibody activity of the immune serum was increased after the immunization. The purified IgM-like immunoglobulin had a tetrameric structure which had a molecular weight of 800 kD and the monomer of IgM-like Ig had a mass of 199 kD which was composed of two heavy chains (Mol. wt. 70 kD) and two light chains (Mol. wt. 29.5 kD). The IgM-like Ig showed hemagglutinating activity to mammalian RBC slightly.**

KEY WORDS: Fish, ***Monopterus albus,*** Immunoglobulin, Molecular structure

It is well known that IgM is the most primitive form of the antibody present in vertebrated because IgM-like immunoglobulin was found in all vertebrates in common and they had some similar characters (Litman, 1976). In the majority of vertebrates, IgM is a pentameric molecule with a molecular weight of 800-1,000 kD (Marchalonis, 1977). In the course of phylogenetic development, the IgM-like immunoglobulin of Placoderm-derived vertebrates exhibited considerable variation in polymer composition, heavy chain length and intersubunit non-covalent bonding. However, other physicochemical properties such as intersubunit disulfide bonding, secondary structure, amino acid composition and electrophoretic heterogeneity were largely unchanged. The limited amount of amino acid sequence data available for low vertebrate IgM-like immunoglobulin heavy and light chain variable region indicate considerable degrees of interspecies homology (Litman, 1982). In fish, two types of IgM-like immunoglobulin was found. A pentameric molecule occurs in Chondrichtyes (shark-like fish) (Fuller *et al.*, 1978; Kobayashi *et al.*, 1982) and Dipnoi (lungfish) (Litman *et al.*, 1971), whereas in Osteichthyes (bony fishes) some tetramer with a molecular weight of 600-800 kD (Acton *et al.*, 1971; Havarstein *et al.*, 1988) and a pentamer (Pollara *et al.*, 1968) were found.

This paper describes the molecular structure of IgM-like immunoglobulin of *M. albus*. The *M. albus*, an interesting fresh water Osteichthyes, have a similar morphological feature to eel (Chondrichthyes with exception of a Brook lamprey), but live only in fresh water during a whole lifetime and have not pectoral fin and ventral fin. We studied IgM-like immunoglobulin in the serum of *M. albus* for the phylogenetic study of immunoglobulin in molecular level.

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## Materials and Methods

### Fish and Immunization

*Monopterus albus* were purchased from a local fish dealer in Seoul and they were kept in the aquarium at ambient temperature ranging 18-20°C until immunization. The fish were immunized with BSA (0.1 mg/0.2 ml/individual) mixed with an equal volume of Complete Freund's Adjuvant. Fourty individuals (five for control) were immunized three times by seven days intervals.

### Extraction of euglobulin and measurement of antibody activity

The blood were collected by decapitation and the sera were fractionated by centrifuation at 1,500g for 20 min. After centrifugation, the euglobulin was salted out with 50% ammonium sulfate (v/v). The precipitate was redissolved in 0.14 M NaCl (final protein conc. of 100 mg/ml), and this euglobulin fraction was used for further isolation of immunoglobulin. In order to measure the antibody activity, a haemagglutination (HA) test was performed using the antigen conjugated human "0" RBC as the target cell. The RBCs were prepared by treatment with tannic acid (0.2% in PBS, pH 7.2) for 30 min at 37°C and the tanned RBCs were sensitized with antigen (BSA in PBS, pH 6.4) for 30 min at 25°C (Bing *et al.,* 1967).

### Chromatographic purification of immunoglobulins

The euglobulin obtained by a salting out procedure was loaded on a DEAE-cellulose column (4.5 × 5.0 cm) which was equilibrated with Tris buffer (20 mM Tris-HCl, pH 8.0), and eluted by a stepwise gradient using Tris buffer containing different molarities of NaCl (0.00, 0.10, 0.13, 0.17 and 0.4 M NaCl). The eluted protein fractions from DEAE-cellulose chromatography were dialyzed with 0.2% NaCl solution (200 vol., twice) and then were concentrated by ultrafiltration using a PM-10 membrane (Amicon Co.). All concentrated protein fractions eluted from the DEAE-cellulose column were tested for their antibody activities by employing the HA test. The fractions containing antibody activities were rechromatographed on Sephacryl S-300 column (2.0 × 130 cm) equilibrated with Tris buffer containing 0.14 M NaCl.

### Molecular weight determination and Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE were done as described by Davis (1964) and Laemmli (1970), respectively. For the measurement of molecular weights of immunoglobulins and of their subunits, SDS-PAGE and gel-filtration were performed. A gel-filtration calibration kit (Pharmacia Co.), electrophoresis calibration kit (Bio-Rad Co. and Pharmacia Co.) and human IgM were used as standards for molecular weight determination.

## Results

### Antibody activity of immune sera

Six animals (five for BSA-immune and one for control) were bled at each immunization step, and Fig. 1. shows their antibody activities at each step. The HA titer of anti-BSA serum to BSA-conjugated RBC increased gradually after immunization. The control (non-immune sera) showed some haemagglutination activity to the RBCs, however the titer was very lower than that of the immune sera.

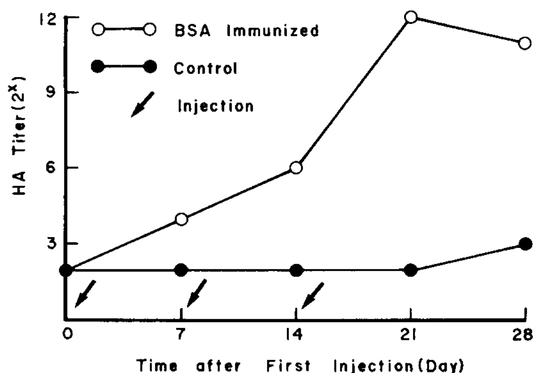


**Fig. 1.** Immune response of *M. albus* to BSA. For HA test, RBC was coated with BSA and was reacted with each antiserum. The innate RBS was used for the control.

**Purification of IgM from anti-BSA serum**

The anti-BSA serum (obtained from the bleedings at 21th and 28th day after first injection) was salted out with ammonium sulfate and the resulting euglobulin was fractionated by DEAE-cellulose chromatography. According to the HA test using BSA-conjugated RBC, it was revealed that the fractions $D_1$, $D_2$ and $D_3$ had antibody activities among the tested fractions. However all fractions still contained some impurities according to electrophoretic analysis (data was not shov .ı).

The fraction $D_1$ was further chromatographed on Sephacryl S-300 column. As shown in Fig. 2, fraction $D_1$ was divided into four subfractions through gel-filtration. By the viture of electrophoretic pattern and HA titer, it was revealed that the antibody positive fraction, $D_1D_1$, showed a single band without impurity (Fig. 2).

The fraction $D_2$ obtained from DEAE-cellulose chromatography was applied to Sephacryl S-300 column (2.0 × 130 cm). The first fraction, $D_2S_1$, had an antibody activity and it contained two protein bands on electrophoretic analysis (Fig. 3). The fraction $D_2S_1$ was divided into two subfractions based on their eluting profiles(left half, $D_2S_{11}$; right half, $D_2S_{12}$ in Fig. 3). The first subfraction showed a single protein band on SDS-PAGE (Fig. 4a), and also had stronger antibody activity than the second subfraction which showed two protein bands on PAGE, respectively. Thus, the first subfraction($D_2S_{11}$) obtained from $D_2S_1$ was used for the characterization of immunoglobulin.

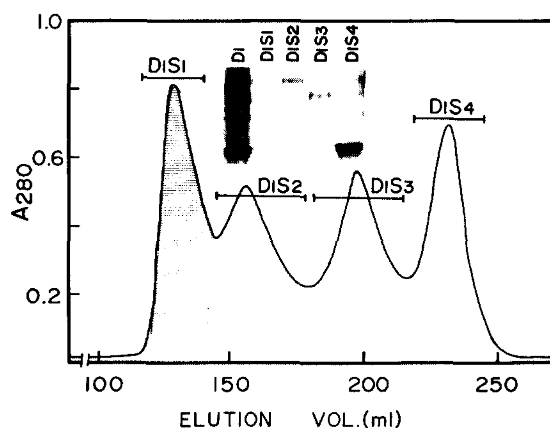


**Fig. 2.** The separation of the fraction $D_1$ from DEAE-cellulose chromatography by Sephacryl S-300 gel filteration. Shaded area and photo represented antibody active fraction and electrophoretic patterns of subfractions (6.0% gel), respectively.

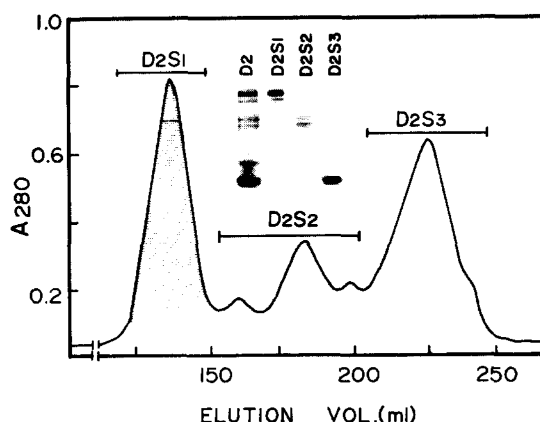


**Fig. 3.** Sephacryl S-300 gel filtration of the fraction $D_2$ which was fractionated by DEAE-cellulose chromatography. Each fraction was analyzed by PAGE (6.0%, photo), and shaded area showed antibody activity based on HA test.

**Molecular structure of IgM-like immunoglobuin**

Purified and concentrated $D_1S_1$ and $D_2S_{11}$ were subjected to SDS-PAGE for their purities. Laemmli's SDS-PAGE system was employed with the slight modification of sample treatment. The 2-mercaptoethanol was deleted from the Laemmli's sample reagent, and the sample was mixed with modified reagent and was incubated for 5 min at 25°C before applying to the SDS-PAGE gel. Fractions $D_1S_1$ and $D_2S_{11}$ showed a single and homogeneous protein band without impurity, and their molecular sizes were identical each other (Fig. 4a). The molecular weight of immunoglobulin $D_1S_1$ (and $D_2S_{11}$) was determined by gel filtration on Sephacryl S-300 (2.0 × 130 cm), and the molecular weight of $D_1S_1$ (also $D_2S_{11}$) was about 800 kD (Fig. 4b). Thus we thought that the purified immunoglobulin ($D_1S_1$ and $D_2S_{11}$) was IgM type immunoglobulin present in the serum of *M. albus*.

In order to elucidate the subunit structure of IgM, 5-12.5 % gradient and 7.5 % gels of SDS-PAGE were carried out. The heat-treated IgM at 70°C was divided into three constituents

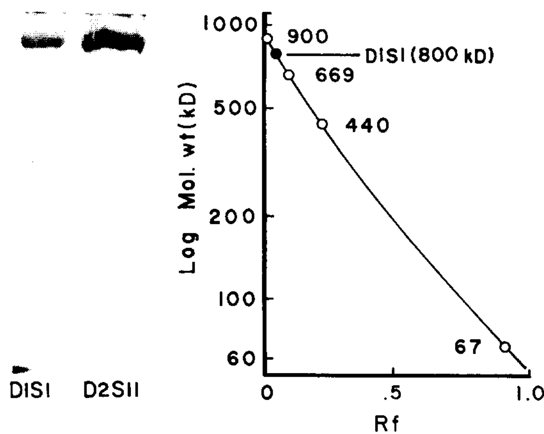


**Fig. 4.** SDS-PAGE (5.5%) pattrns of $D_1S_I$ and $D_2S_{II}$ and the calibration curve using standards for mol. wt. determination. $D_1S_I$ and $D_2S_{II}$ were not treated with 2-MSH for electrophoresis and the $R_f$ of $D_1S_I$ was the average value of three determinations of gel filtration.

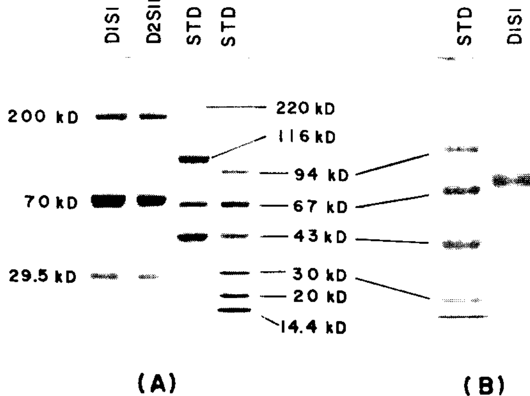


**Fig. 5.** SDS-PAGE analysis of IgM-like immunoglobulin presented in the serum of *M. albus* with standard proteins. (A) 5-12.5% gel SDS-PAGE, the samples were heat-treated for 3 min at 70°C. (B) 7.5% gel SDS-PAGE, the samples were heat-treated for 3 min at 100°C.

which had molecular weight of 200 kD and 70 kD and 29.5 kD, respectively (Fig. 5a). However, when the IgM-like molecule was treated at 100°C, it was divided into two constituents of 70 kD and 29.5 kD (Fig. 5b). Based on these results (Fig. 5), it was possible to demonstrate the general features of heavy and light chains present in the purified IgM. The protein bands of 70 kD and 29.5 kD may be considered as heavy and light chain respectively, and the protein band of 200 kD may be polymerized form of heavy chain and light chain.

The antibody positive fraction $D_3$, obtained from DEAE-cellulose chromatography, was also analyzed according to the same chromatographic procedures described above. The specific antibody positive IgM-like immunoglobulin was absent and small molecular weight antigen specific protein (about 120 kD) was detected (data was not shown).

## DISCUSSION

As judged by the results of Fig. 4 and Fig. 5, the IgM-like Ig of *M. albus* may be organized into four monomers (4 × 199 kD) and the monomer was composed of two heavy chains (2 × 70 kD) and two light chains (2 × 29.5 kD). Therefore, it was possible to elucidate that the molecular weight of IgM-like Ig of *M. albus* was about 796 kD and it's structural formula was $(H_2L_2)_4$ type. The whole mol. wt of IgM-like Ig of *M. ablus* was about 800 kD by gel-filtration and was 796 kD by structural formulation based on electrophoretic data. The difference between 800 and 796 may be either methodological difference or the effect of other attendant subunit such as joint chain. Chondrichthyes (horned shark, lemon shark: Clem and Small, 1967), Chondrostei (paddle fish: Pollara *et al.*, 1968) and dipnoi (Australian lungfish: Marchalonis, 1969) have a pentameric IgM-like molecule, and Holostei (bowfin: Litman, 1971) and Teleostei (chumsalmon: Kobayashi, 1982) have a tetrameric immunoglobulin. The IgM-like Ig of *M. albus* (Order Synbranchida) has a tetrameric structure which was coincided with other Osteichthyes. The fish have similarity in mass of subunits of IgM. Their heavy chains of the fish IgM consistently exhibit molecular weights of about 70 kD and the light chains exhibit molecular weight of 21-24 kD. The H-chain mass of IgM in the serum of *M. albus* was similar to that of other fish, however the light chain had a molecular weight of 29.5 kD which was considerably greater than that of other fish as described above.

As well as immune sera, the normal serum from

*M. albus* also showed HA titer by 4-8 fold, although the titer was lower than that of immune serum (Fig. 1). These results indicated that some kinds of natural agglutinin may be existed in the serum of *M. albus*. However, through the purification process, it was proved that the HA activity was originated from IgM-like immunoglobulin.

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**드렁허리(*Monopterus albus*) 혈청내 IgM유사 면역글로불린의 구조적 특성**

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BSA로 면역시킨 드렁허리의 혈청에서 IgM 유사면역글로불린을 정제한 후 그 구조적 특성을 구명하였다. 면역후 항혈청에서는 항원특이 항체능이 증가되었다. 정제된 IgM 유사면역글로불린은 199 kD의 분자량을 갖는 단량체 4개가 결합된 800 kD의 단백질이었으며, 단량체는 70 kD인 H-사슬 2개와 29.5 kD인 L-사슬 2개가 결합된 형태였다. 또한 드렁허리의 IgM 유사면역글로불린은 IgM의 특성과 같이 포유류 적혈구에 대하여 약한 응집력을 나타냈다.