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ISOLATION AND CHARACTERIZATION OF THE IMMUNOGLOBIN OF PIKE (ESOX LUCIUS L.)

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ABSTRACT

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The purification of the immunoglobulin from pike serum and its physicochemical characterization is presented. The immunoglobulin was prepared by means of gel filtration and ion exchange chromatography. Measurements in the analytical ultracentrifuge showed a sedimentation constant of 15.0 S. A molecular weight of 650.000 was calculated. The immunoglobulin was composed of heavy and light chains of molecular weights 60.000 and 22.500, respectively. It is likely that the immunoglobulin of pike is composed of 8 heavy and 8 light chains and possesses a tetrameric structure. The heavy chains contain 9.2% sugars and amino sugars. The amino acid composition of the chains is similar to that of other fish immunoglobulins.

INTRODUCTION

The ability to synthesize humoral antibody in response to antigenic stimulation is possessed by representatives of all vertebrates (Ambrosius, 1976; Atwell and Marchalonis, 1976; Benedict and Yamaga, 1976; Du Pasquier, 1976; Litman, 1976; Manning and Turner, 1976; Rowlands, 1976). True antibody responses have been observed on a phylogenetic level as low as that of the chondrichthyes (Clem and Small, 1967; Marchalonis and Edelman, 1965; Suran et al., 1967). Chondrichthyes apparently have only one class of immunoglobulin which occurs in both pentameric and monomeric form. Bony fishes also have only one class of immunoglobulin which usually is of tetrameric nature.

In spite of their economic importance, little physicochemical and biological data are available for the immunoglobulins of the numerically largest group of osteichthyes (Clem, 1971; Litman, 1976; Manning and Turner, 1976). In the light of paucity of data on immunoglobulins of bony fishes, the finding that pike serum yielded appreciable amounts of antibody against pike fry rhabdovirus (Clerx et al., 1978) and the relative ease of

collecting large amounts of blood from pike, a further characterization of pike immunoglobulin was undertaken.

METHODS

Animals. Pike were captured at Westbroek, province of Utrecht, the Netherlands. Bleeding was performed in the laboratory after anaesthesia with MS-222 (Sandoz, Basle, Switzerland) as described previously (Clerx et al., 1978). Blood was allowed to clot at room temperature and the serum was harvested after centrifugation for 30 min at 1200 x g (4°C). Pooled serum was stored at -20°C.

Sera. The preparation of pike anti-pike fry rhabdovirus serum has been described elsewhere (Clerx et al., 1978). Rabbit antisera to pike whole serum and to purified reduced heavy chains of the immunoglobulin were prepared by injecting either 0.5 ml or 1.5 - 2 mg of the respective antigens emulsified in Freund's completer adjuvant interdigitally, intramuscularly and subcutaneously. The animals received a second dose of antigen in incomplete adjuvant by intramuscular and subcutaneous route two weeks later. Antisera were obtained 2-3 weeks after the last injection.

Reagents. All chemicals used in this study were reagent grade or the best grade commercially available. Guanidine hydrochloride was obtained from Sigma (St. Louis, Mo., USA). It was clarified by passage of a 6 M solution through a 0.2 m membrane filter. This resulted in a solution with an absorbance at 280 nm of less than 0.02/cm.

Preparation of immunoglobulin. Pike serum was freed from lipoproteins as described by Bourne (1969). Serum proteins were precipitated three times by dropwise addition of ammonium sulphate till 40% saturation. Proteins were allowed to flocculate for at least 3 h at 4°C. Pellets obtained by centrifugation at 10.000 x g for 30 min at 4°C, were dissolved in gel filtration buffer (1.0 M NaCl, 0.1 M tris-HCl pH 8.0 containing 0.02% sodium azide). After the third precipitation the pellets were dissolved in an amount of buffer corresponding to 15-20% of the original serum volume. Before application to columns the preparation was dialyzed for 18-24 h against a 100-fold excess of buffer.

Precipitates were then removed by centrifugation at 10.000 x g for 10 min. Ten to fifteen milliliters of the clear supernatant were applied to Ultrogel AcA 22 columns (LKB Produkter, Stockholm, Sweden; tandem columns, 100 x 2.5 cm each) using a four-way valve and a flow adapter (Pharmacia Benelux, The Hague, The Netherlands). The flow rate was 15-16 ml/h and fractions of 3-9 ml were collected with the aid of an Ultrorac 7000 fraction collector with continuous absorption measurement at 280 nm (Uvicord type 8301, LKB). The purity of the column fractions was subsequently checked by SDS-polyacrylamide electrophoresis of 0.005 - 0.01 ml samples (Laemmli, 1970). Column fractions containing minor amounts of contaminating protein were pooled and the immunoglobulin precipitated with ammonium sulphate as described above. Further purification was achieved by ion exchange chromatography on DE 52 cellulose (Whatman) or DEAE-Sephadex A-50 (Pharmacia) columns. Since the immunoglobulin precipitated at low salt concentrations, urea was added to the buffers for ion exchange chromatography (Clem, 1971). Extensive dialysis of immunoglobulin against starting buffer (0.25 M urea in 0.01 M phosphate buffer pH 7.0) strongly influenced the binding of the protein to the column (see RESULTS). However, contaminating proteins were removed more efficiently when dissolved immunoglobulin was immediately subjected to chromatography. Elution of bound proteins was performed with linear NaCl gradients, the limit buffer being 0.1 M in NaCl. After extensive dialysis against the urea containing buffer, the NaCl concentration had to be raised to 0.3 M in order to elute all bound protein. Ion exchange chromatography column fractions were checked for contaminants by SDS-polyacrylamide electrophoresis and immunoelectrophoresis using anti-pike whole serum.

Isolation of immunoglobulin chains. Dissociation and reduction of purified immunoglobulin was performed in 8 M guanidine-HCl and 0.5 M mercaptoethanol at pH 8.0 for 4 h at 50°C (Green and Bolognesi, 1974). Alternatively, oxidative sulfitolysis in 8 M guanidine-HCl was used to cleave inter- and intra-chain disulfide bridges as described by Edelman and Marchalonis (1967). After dialysis against column buffer (6 M guanidine-HCl, 0.05 M sodium acetate buffer pH 5.0, 0.02 M mercaptoethanol) reduced or sulfitolyzed chains were separated on a 100 x 2.5 cm Sepharose 6B-CL column (Pharmaica) equilibrated with the same buffer. Samples contained 20-100 mg protein. The flow rate was regulated at

1-2 ml/h by a peristaltic pump. After monitoring the column effluent at 280 nm, pooled fractions of heavy (H) and light (L) chains were extensively dialyzed against distilled water with (reduced chains) or without 0.002 M mercaptoethanol (sulfitolyzed chains) and lyophilized (Edelman and Marchalonis, 1967; Green and Bolognesi, 1974). Purity of isolated chains was checked by SDS-polyacrylamide electrophoresis.

Physical measurements. Analytical ultracentrifugation was conducted as described for similar studies on lemon shark immunoglobulins (Clem and Small, 1967). The partial specific volume ($\bar{v}$) of the immunoglobulin was calculated from the amino acid and sugar composition of isolated chains assuming a 1:1 ratio of heavy and light chains and carbohydrate $\bar{v} = 0.61$ (Clem, 1971; Cohn and Edsall, 1943). The molecular weight of heavy chains was determined by sedimentation equilibrium centrifugation (Heytink, 1974; Heytink et al., 1977). The $\bar{v}$ of heavy chains in 5.0 M guanidine hydrochloride was determined in the Precision Density Meter DMA 02C of Anton Paar (Austria).

Carbohydrate determinations. These were performed in duplicate on purified heavy chains as described previously (Kamerling, et al., 1975).

Amino acid analyses. Analyses were performed on a Chromaspek automatic amino acid analyzer utilizing a 35 x 0.26 cm column as described by Murren et al., (1975). Samples of pure chain preparations were hydrolyzed in 6.0 M hydrochloric acid under vacuum at 110°C for 24 hours. Norleucine was used as a standard. Proline was determined in duplicate. Values for the other amino acids are the averages of four determinations. Serine, threonine and valine values are corrected for losses due to acid hydrolysis (Kraal, 1972; Kraal et al., 1972).

SDS-polyacrylamide electrophoresis. Apparent molecular weights of immunoglobulin chains in polyacrylamide gels were determined using the gel system of Laemmli (1970), either in cylindrical gels or in a slab gel aparatus (Studier, 1973). In some instances the gel system of Maizel (1971) was used for light chains. Electrophoresis in alkaline urea gels was performed in the slab gel apparatus. These gels were prepared according to Reisfeld

and Small (1966). Stained gel strips from slab gels or cylindrical gels were scanned at 580 nm in an ISCO Gel Scanner model 1310 (CENCO, Breda, the Netherlands). Marker proteins for SDS-polyacrylamide electrophoresis of 94,000, 68,000, 60,000, 53,000, 43,000, 40,000, 25.700, 24.500, 21.500, 20,000, 17,000, 14.300, and 11.300 daltons were used.

Protein determinations. Purified immunoglobulin was estimated by absorbance at 280 nm. The absorbance of a 1% solution was found to be 13.1 $\pm$ 0.1 / cm by comparison with the Folin-Ciocalteu (Lowry et al., 1951) and biuret determinations. Protein concentrations of more than 2 mg/ml were usually determined employing the biuret procedure. Lower concentrations were determined by the method of Lowry. Bovine serum albumin was used for the construction of calibration curves.

Immunoelectrophoresis. Immunoelectrophoresis was performed on 7.5 x 2.5 cm glass slides at 200 V for 1.5 h as described earlier (Grabar and Williams, 1953; Scheidegger, 1955).

RESULTS

Purification. A typical elution pattern of pike serum proteins from Ultrogel AcA 22 columns is shown in Fig. 1. Fractionation of ammonium sulphate precipitated proteins resulted in one major and several minor peaks. Similar patterns were obtained when using Sephadex G-200, Sephacryl S-200 or Sepharose 6B columns. The immunoglobulin recovered in the major peak of the latter columns, however, showed at least 3 precipitin lines when tested against anti-pike whole serum in immunoelectrophoresis slides.

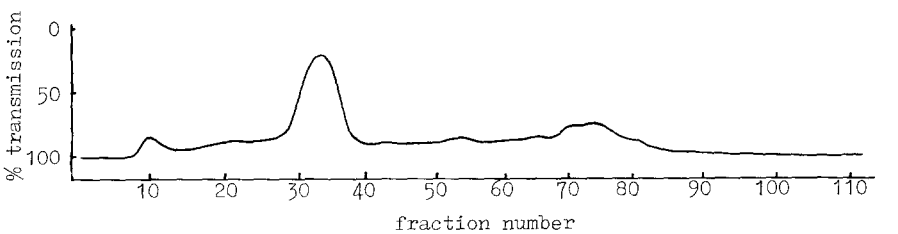

Figure 1. Elution pattern of ammonium sulphate precipitated pike serum proteins on tandem Ultrogel AcA 22 columns. Original serum volume was 44 ml. One fraction is 150 drops.

Presence of the immunoglobulin in the major peak was demonstrated by testing column fractions for virus neutralizing activity (Clerx et al., 1978). Gel filtration of serum collected 613 days (after 15 injections with pike fry rhabdovirus) showed that the immunoglobulin was confirmed to one high molecular weight class. SDS-polyacrylamide electrophoresis of column fractions revealed the presence of heavy and light polypeptide chains in the virus neutralizing fractions. Since it was much easier to detect immunoglobulin chains in SDS-polyacrylamide gels that to test column fractions for virus neutralizing activity, slab gel electrophoresis was routinely used for the detection of immunoglobulin (Fig. 2). SDS-polyacrylamide electrophoresis proved to be more sensitive in the detection of contaminants than immunoelectrophoresis using our anti-pike whole

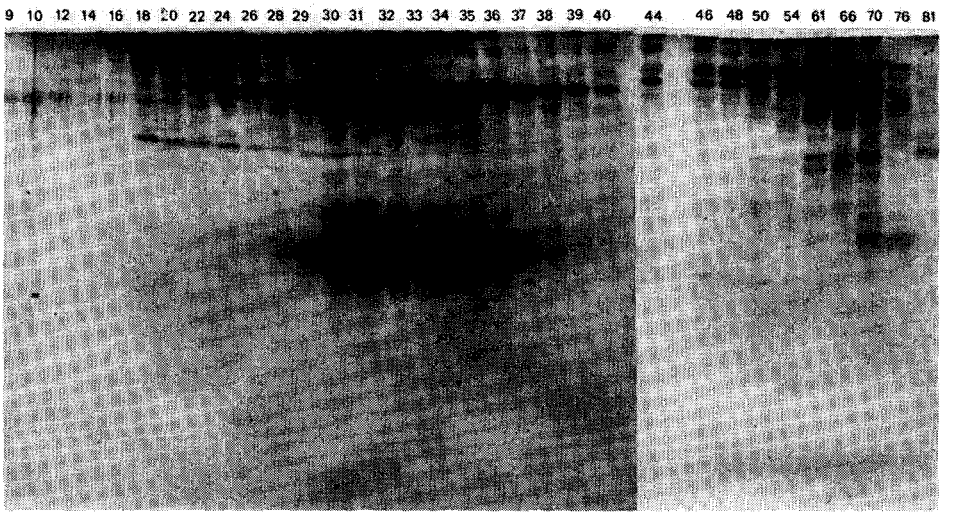

Figure 2. SDS-polyacrylamide slab gel electrophoresis of the column fractions from Fig. 1. The stained gels show the coincidence of absorbance in Fig. 1 with the presence of heavy and light chains upon electrophoresis, as well as the distribution of contaminating proteins. The absence of a low molecular weight antibody is evident. Electrophoresis (at 100 V for 3 h) was performed with SDS-mercaptoethanol disintegrated 0.01 ml samples in 11% gels.

serum: column fractions showing one precipitin line upon diffusion against the antiserum often contained up to three contaminating polypeptides in slab gels. Fractions of Ultrogel AcA 22 columns containing more than 95% immunoglobulin (as judged from stained slab

gels) were used for further studies. Initial efforts to purify the immunoglobulin by ion exchange chromatography were hampered by its insolubility in the low ionic strength buffer employed. However, after the addition of urea to the buffer the preparation was completely soluble. Figs. 3 and 4 show the elution patterns of a DEAE-cellulose column separation of the immunoglobulin peak from Fig. 1. Contaminating protein species bound

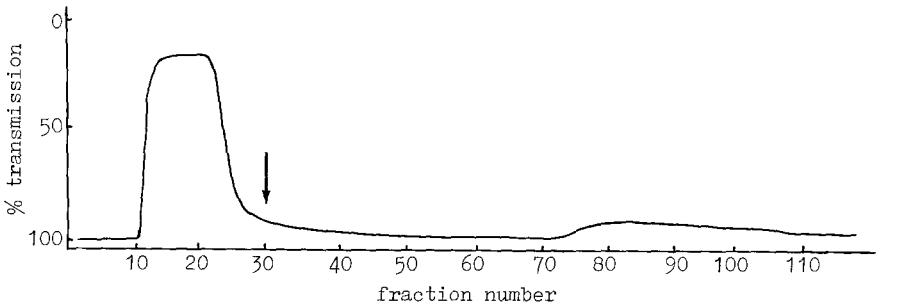

Figure 3. Cellulose ion exchange chromatography. Precipitated IgM from fractions 30 to 36 of Fig. 1 was dissolved in 25 ml starting buffer and chromatographed on a 35 x 2.5 cm DE 52 column. Elution was performed with a 0 - 0.1 M NaCl gradient. The arrow indicated the start of this gradient. The flow rate was maintained at 25 ml/h. One fraction is 75 drops.

to DEAE eluted at NaCl concentrations of 0.06 - 0.08 M (Fig. 3), whereas all immunoglobulin appeared in the excluded volume. After overnight dialysis of the excluded immunoglobulin against the starting buffer several peaks were observed in the subsequent chromatography (Fig. 4) which were probably due to the unfolding of the

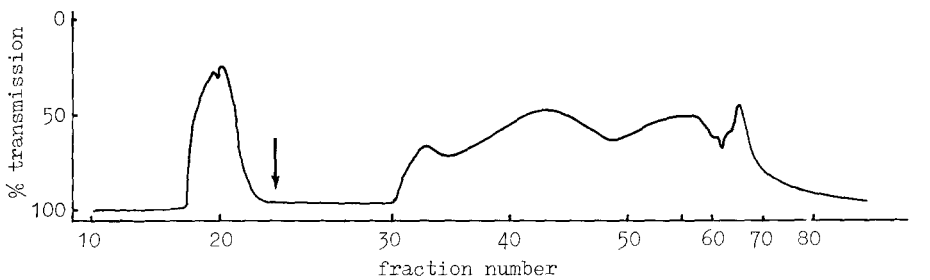

Figure 4. DE 52 chromatography of fractions 11 to 26 from Fig. 3 (concentrated to 10 ml by ammonium sulphate precipitation) after overnight dialysis against starting buffer. The arrow indicates the start of a 0 - 0.3 M NaCl gradient. The flow rate was 20 ml/h; at fraction 56, the flow rate was increased to 50 ml/h. Gradient volume was 400 ml. One fraction is 75 drops.

immunoglobulin by the urea. Polyacrylamide electrophoresis showed that all peaks contained polypeptides of identical molecular weight (Fig. 5). Immunoelectrophoretic analysis of the column fractions also indicated that they were free from contaminating proteins. When the fractions were reacted with rabbit anti-pike whole serum, a single precipitin arc developed in contrast to the immunoglobulin peak from the Ultrogel column which revealed one major and two minor precipitin arcs (results not shown).

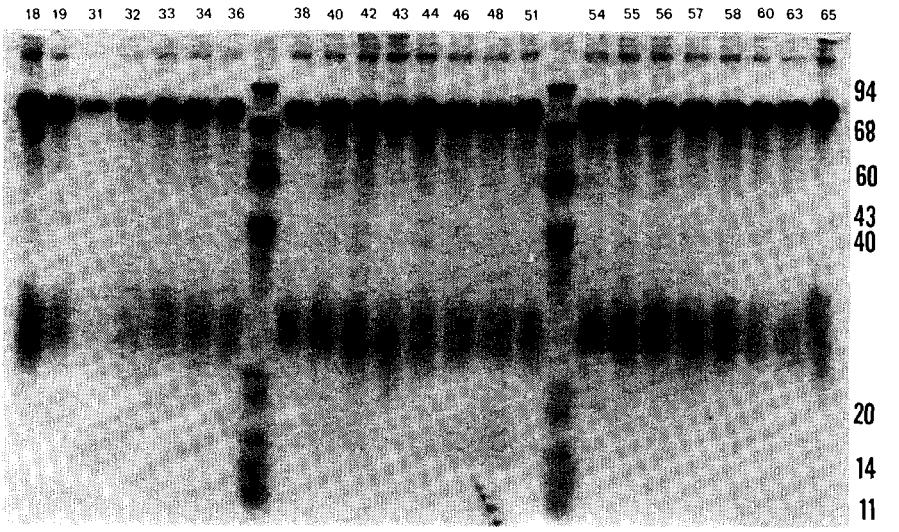

Figure 5. Slab gel electrophoresis of ion exchange chromatography fractions. Fractions of the major peaks in Fig. 4 were electrophorezed in 10% gels for 2½ h at 100 V. Marker proteins (94,000, 68,000, 60,000, 43,000, 40,000, 20,000, 14.300 and 11.300 daltons, scale at the right) were applied to the slots between column fractions 36/38 and 51/54, respectively.

Immunoglobulin purified by gel filtration on tandem Ultrogel AcA 22 columns and subsequent DEAE-cellulose ion exchange chromatography will be referred to as purified IgM.

Physical measurements. The sedimentation pattern of purified IgM in the ultracentrifuge showed a fairly symmetrical Schlieren peak (not shown). During centrifugation a protein sediment accumulated at the bottom of the cells. Using three concentrations of purified

IgM (10, 5 and 2 mg/ml in 0.01 M phosphate buffer, pH 7.2), a $S_{20,w}$ of 15.0 was calculated at infinite dilution. Diffusion coefficient determination by measuring peak broadening during low speed centrifugation and Schlieren optics did not give a diffusion coefficient in agreement with a high molecular weight molecule. Preliminary laser-beat measurements, however, showed that the coefficient was concentration independent (Dr. H. Mos, personal communication). When using diffusion coefficient values measured for the immunoglobulins of other fish species (Acton et al., 1971a) a molecular weight between 638.000 and 651.000 daltons is obtained for the pike immunoglobulin. Attempts to prepare subunits from the microglobulin were without success. Pike IgM proved to be very resistant against conditions known to produce subunits of mammalian IgM (Edelman and Marchalonis, 1967). Reduction with 1 M mercaptoethanol or 0.2 M dithiotreitol in 8 M urea (60 min at 25°C) and subsequent alkylation with iodoacetamide or iodoacetic acid failed to produce H_2L_2 subunits. Chromatography of such samples on columns in 8 M urea showed only one major peak as well as a small amount of free light chains (results not shown). Studies in the ultracentrifuge using reduced or unreduced samples layered on 8 M urea or 2-8 M guanidine hydrochloride with or without 0.05 M mercaptoethanol, showed a similar picture: light chains were liberated under all conditions, whereas no monomeric subunits or liberated heavy chains could be found in the supernatant under conditions where only the aggregated form would reach the bottom. Bovine IgG on 8 M urea remained in the upper half of the tube after centrifugation.

Antigenic studies. Ouchterlony double diffusion tests showed that the antiserum raised against isolated heavy chains (see below) reacted with polymeric immunoglobulin (Fig. 6). A reaction of partial identity was observed between the antigen and IgM. The complete identity observed between pike IgM and IgM of another fish, namely carp, suggests that a limited number of antigenic determinants on IgM are recognized by the anti-heavy chain serum. This is supported by the lack of reactivity with grass carp serum, since antiserum to carp IgM does cross-react with grass carp IgM (Dr. W.B. van Muiswinkel, personal communication). The double precipitin line observed with pike and carp IgM suggests that at least two antigenic determinants may be involved. The antigenic determinants recognized by the anti-heavy chain serum must therefore be present on carp IgM, but not

on grass carp IgM.

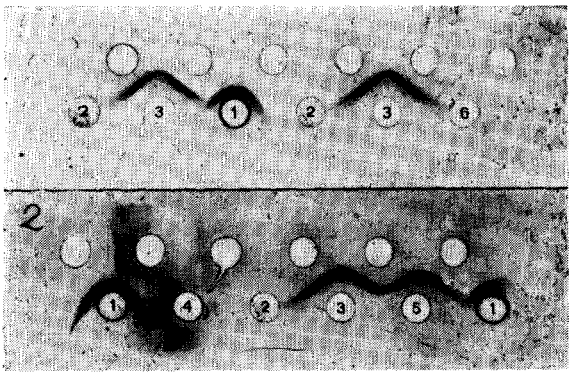

Figure 6. Ouchterlony double diffusion test with rabbit antiserum to purified heavy chains of pike IgM (upper wells of each slide) against (lower rows): carp and grass carp serum, purified pike IgM, human IgM and isolated chains of pike IgM. Wells designated 1 = pike IgM heavy chains, 2 = pike IgM light chains, 3 = pike IgM, 4 = human IgM, 5 = carp serum, 6 = grass carp serum. Double precipitin lines which show complete identity are formed between pike IgM and carp serum. A reaction of partial identity is observed between purified heavy chains and pike IgM (top left).

Studies on heavy and light polypeptide chains. The carbohydrate composition of purified heavy chains (see below) is shown in Table I. The values reported are the averages of duplicate determinations. Moles/mole protein were calculated using a molecular weight of 60.000 daltons for the heavy chain (see below). Using the anthrone reagent, no carbohydrate could be detected in light chain preparations.

TABLE I

Carbohydrate composition of purified immunoglobulin heavy chains

	% CHO	moles/mole protein
fucose	0.28	1.0
mannose	1.81	6.0
galactose	1.78	5.9
glucosamine	2.65	7.2
galactosamine	1.15	3.1
sialic acid	1.55	3.0
total CHO, %	9.22	
mannose/galactose ratio		1.02

Following extensive reduction or oxidative sulfitolysis in 8 M guanidine hydrochloride the heavy and light chains were separated by gel filtration on Sepharose 6B-CL in the presence of 6 M guanidine hydrochloride as shown in Fig. 7. The heavy and light

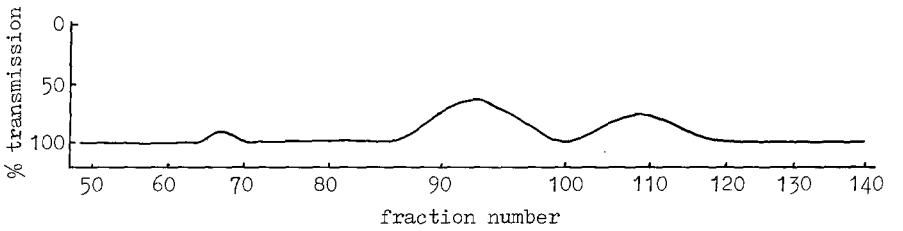

Figure 7. Gel filtration of sulfitolyzed IgM chains in 6 M guanidine hydrochloride in 0.05 M sodium acetate buffer pH 5.0 containing 0.02 M mercaptoethanol (Green and Bolognesi, 1974). Gel matrix: Sepharose 6B-CL; column dimensions: 90 x 2.5 cm, downward flow regulated at 1.5 ml/h. Fractions 88 to 97 and 103 to 117 were pooled as heavy and light chains, respectively.

chains were pooled as indicated in the legend of the figure and examined by SDS-polyacrylamide electrophoresis. Light chains did migrate heterogeneous in the gel system of Laemmli (Fig. 8). However, when using the gel system formulated by Maizel (1971), migration was uniform. As can be seen in Fig. 9, the chains are homogeneous with respect to size and they do not contain detectable amounts of the other chain.

Heavy chains from disintegrated IgM or isolated heavy chains migrate in SDS-polyacrylamide gels as having apparent molecular weights of 70.000 to 72.000 daltons (Fig. 8 and 10). No dependence of the molecular weight from the polyacrylamide concentration was observed at concentrations between 7 and 15%. As can be seen in Fig. 10, pike IgM heavy chains migrate identical to or slightly faster than their mammalian counterpart.

In slab gels prepared according to Laemmli (1970) light chains migrate as more or less discrete bands (Fig. 8 and 10). This heterogenicity in light chain migration could be attributed to differences in charge since migration in gels at pH 9.2 is much more uniform than at pH 8.3 (Fig. 9). The molecular weight of pike light chains is similar to that of human light chains (22.500 daltons, Fig. 10). The extrapolated value of 22.500 of pike light chains was in good agreement with marker proteins of 25.700, 24.500, 21.500, 20.000 and 17.000 daltons as references.

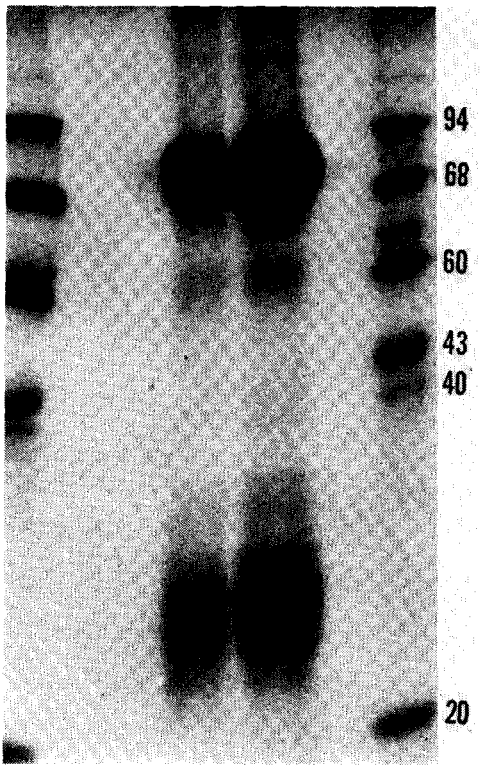

Figure 8. SDS-polyacrylamide electrophoresis of purified pike IgM (central lanes). The broad area between 28,000 and 20,000 daltons at which light chains have migrated, shows their heterogeneous mobility in the Laemmli gel system. Marker proteins are shown at right.

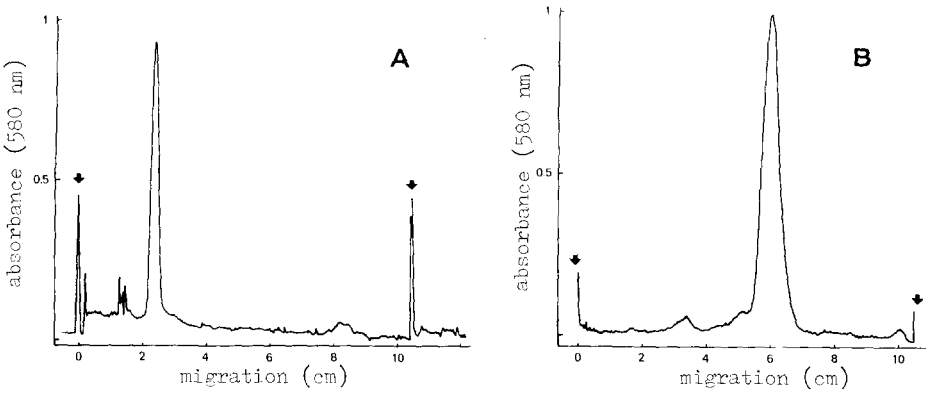

Figure 9. Densitographs of heavy and light chain pools after guanidine hydrochloride gel filtration. Heavy chains (A) were electrophorezed in 11% polyacrylamide gels as formulated by Laemmli (1970). Samples of the light chain pool (B) from Fig. 7 were electrophorezed in 10% cylindrical gels using the gel system formulated by Maizel (1971). The small peak at 3.5 cm migration distance in B probably represents light chain dimers (molecular weight 46-47,000 daltons).

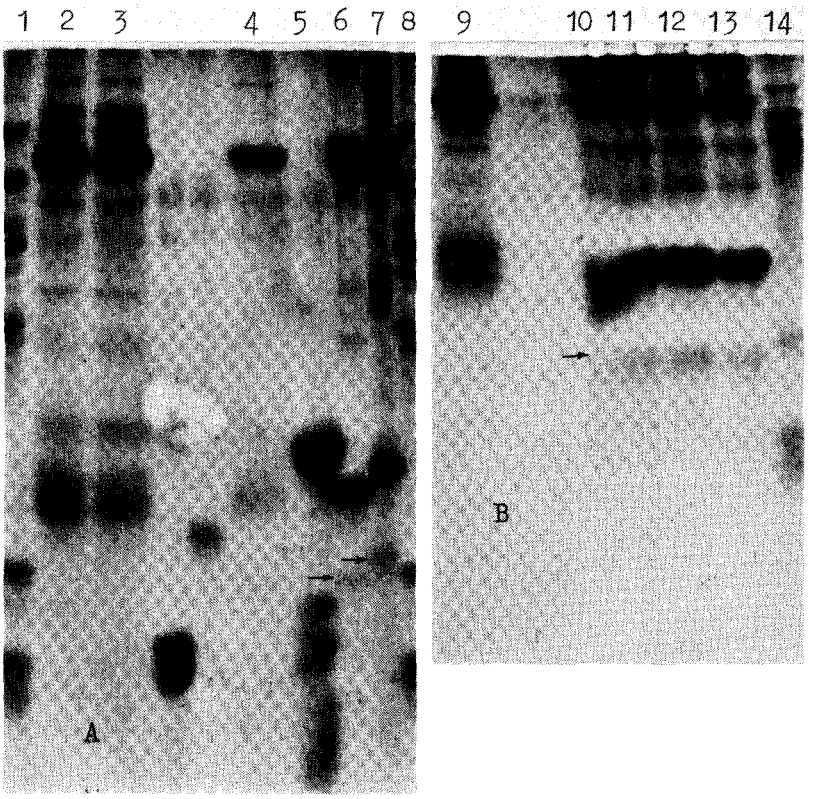

Figure 10. Comparative electrophoresis of pike, dog and human IgM in Laemmli gels at 11% (A) and 15% (B) polyacrylamide concentrations. At both gel concentrations the presence of mammalian J chains is evident (arrows). Lanes 2, 3, 4, 9 = partially purified pike IgM, lanes 1, 8, 14 = marker proteins (94,000, 68,000, 60,000, 53,000, 43,000, 40,000, 20,000 and 11.300 daltons), lane 5 = chymotrypsin (25.600 daltons), lane 6 = dog IgM, lanes 7, 10, 11, 12, 13 = human IgM. Both gels were electrophorezed for 3 h at 100 V.

Mestecky et al., (1975) detected a J chain in the channel catfish high molecular weight immunoglobulin by electrophoresis in alkaline urea gels. No such chain could be detected in pike IgM when either reduced or sulfitolyzed light chain preparations were electrophoresed under conditons (Reisfeld and Small, 1966) where a freshly reduced human IgM sample showed a J chain (Fig. 11). Furthermore, comparative electrophoresis of pike IgM, human IgM and dog IgM in Laemmli gels (Laemmli, 1960) gave no indication of the presence of a third type of immunoglobulin chain in pike IgM while such a chain was observed in the mammalian IgMs (Fig. 10).

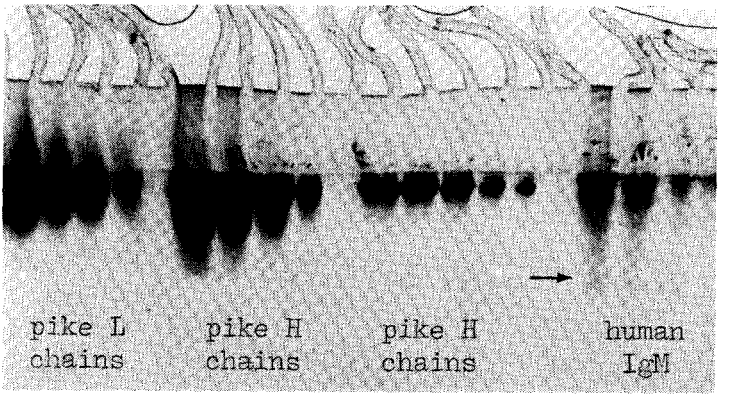

Figure 11. Alkaline urea gel electrophoresis according to Reisfeld and Small (1966) using isolated chains from pike IgM and human polymeric IgM. The photograph shows the absence of a fast moving component in the light chain pool (from Fig. 7) and its presence in human IgM. Samples were heated for 15 min at 45°C in 10 M urea and 10% mercaptoethanol before application to the gel slots. Samples at the left side of the gel were sulfitolyzed before separation on guanidine hydrochloride columns. The arrow indicates the position of the human J chain. Electrophoresis was carried out for 3½ h at 100 V.

Previously it has been suggested that the molecular weight of the heavy chain of immunoglobulins from bony fishes is approximately 70.000 daltons (Acton et al., 1971a, 1971b; Bradshaw et al., 1971; Clem, 1971; Litman et al., 1971a, 1971b, 1971c; Litman, 1976; Marchalonis, 1971; Pollara et al., 1968). These data were obtained from standardized gel filtration columns in guanidine hydrochloride or by polyacrylamide electrophoresis. Using 70.000 daltons for the heavy chain, the aggregate chain weight was always excessive high when compared to the observed molecular weight of intact immunoglobulin molecules. Lower heavy chain weights of bony fish immunoglobulins, however, were obtained when using sedimentation equilibrium analysis in guanidine hydrochloride or other techniques (Acton et al., 1971b; Hall et al., 1973). Therefore, isolated heavy chains of pike IgM were subjected to sedimentation equilibrium centrifugation in 5 M guanidine hydrochloride as described (Yphantis, 1964). Sedimentation equilibrium studies at two sample concentrations gave linear plots of ln fringe displacement (y) versus the radius squared (Fig. 12). The slopes were concentration independent. Correlation coefficients of experimental points to the calculated plot were between 0.99 and 0.9998, indicating that heterogeneity was minimal and no material of higher molecular weight was present.

Using the measured partial specific volume of 0.736 ± 0.05 ml/g in the same solute, a weight average molecular weight of 60.000 daltons was calculated for the heavy chain of pike IgM.

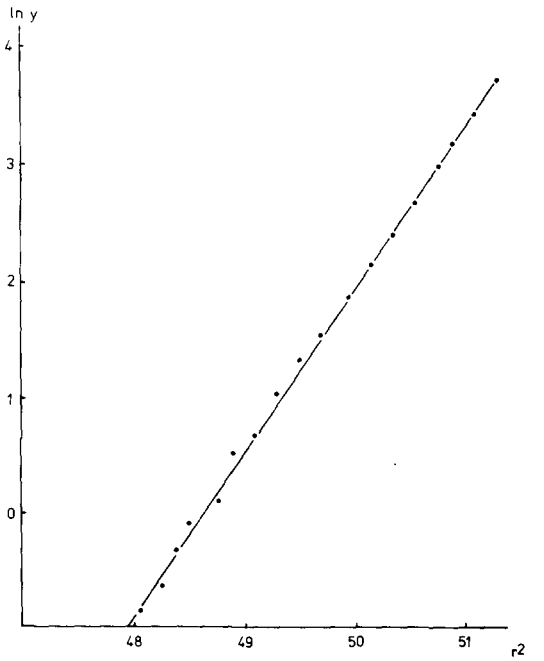

Figure 12. Molecular weight determination of purified heavy chains from pike IgM by the sedimentation equilabrium sedimentation technique described by Yphantis (1964). The figure shows a plot of the logarithm of the protein concentration (fringe displacement) versus distance from the rotor axis squared. Protein concentration of starting material was 0.15 mg/ml. Centrifugation was performed at 23.724 rpm for 63 h at 25°C in an An-F rotor.

The amino acid composition data for the purified heavy and light chains are given in Table II. These values are the average of 4 determinations and are corrected for losses due to acid hydrolysis. Proline values are the average of 2 determinations. The pike immunoglobulin chains have a high content of threonine, serine and glutamic acid. Heavy chains show a high content of lysine, and light chains contain somewhat more glycine than found in other vertebrate immunoglobulin light chains (Acton et al., 1971a, 1971b, 1971c, 1972a, 1972b; Marchalonis and Edelman, 1966; Wilde and Koshland, 1973).

TABLE II

Amino acid composition of pike heavy and light immunoglobulin chains expressed as grams per 100 g of carbohydrate free protein. N. D. = not determined

	H chain	L chain
aspartic acid	9.9	8.3
threonine	10.7	10.9
serine	9.3	13.3
glutamic acid	12.3	11.9
proline	5.4	5.3
glycine	5.8	9.9
alanine	4.2	4.2
cystine (half)	1.1	1.8
valine	7.2	7.4
methionine	2.2	0.5
isoleucine	3.9	2.5
leucine	6.5	8.4
tyrosine	4.5	3.6
phenylalanine	4.1	2.3
histidine	2.4	1.2
tryptophan	N.D.	N.D.
lysine	8.2	5.4
arginine	2.4	3.0

DISCUSSION

The studies presented here describe the characterization of the macroglobulin of pike (Esox lucius L.), a member of the order Salmoniformes. Purification of pike antibodies resulted in the isolation of a single class of molecules. Following long term immunization against pike fry rhabdovirus, antibody activity could be demonstrated only in a high molecular weight component of the serum, as described for a number of other fishes (Acton et al., 1971a, 1971b; Bradshaw et al., 1971; Dorson, 1972; Marchalonis, 1971; Pollara et al., 1968; Shelton and Smith, 1970). Limited data on pike serum obtained by gel filtration and analytical ultracentrifugation have been published some years ago (Gibb et al., 1974). We were able to obtain the immunoglobulin in a highly pure state as judged by sedimentation analysis, immunoelectrophoresis and SDS-polyacrylamide electrophoresis. Aggregation of purified IgM was found to occur rapidly. The molecular weight of 640.000 - 650.000 daltons obtained for the pike immunoglobulin is considerably lower than that for IgM from mammals, birds, reptiles, amphibians and chondrostean fishe s (Litman, 1976). These animals have pentameric macroglobulins in the weight range

of 800.000 - 900.000 daltons as determined by sedimentation equilibrium studies (Clem and Small, 1967; Leslie and Clem, 1969; Marchalonis and Edelman, 1966). However, the calculated value for pike IgM is in good agreement with the molecular weights obtained for bony fish immunoglobulins. From the literature it is apparent that bony fishes usually have only one class of immunoglobulins which is tetrameric rather than pentameric (Acton et al., 1971a, 1971b; Bradshaw et al., 1971; Clem, 1971; Hall et al., 1973; Litman et al., 1971a; Litman, 1976; Marchalonis, 1971; Pollara et al., 1968; Shelton and Smith, 1970).

The carbohydrate composition of the heavy chain of pike immunoglobulin reveals a large amount of galactosamine, an amino sugar hiterto only found in catfish immunoglobulin (Hall et al., 1973). When expressed as percentage of aggregated chains, pike immunoglobulin contains 6.7% carbohydrate, very close to the values found in paddlefish (Acton et al., 1971b) and dogfish (Marchalonis and Edelman, 1966). The ratio of mannose to galactose was found to be 1.0, in agreement with previously published ratios found in fish immunoglobulins (Acton et al, 1971b, 1972; Frommel et al., 1971).

Following extensive reduction as described by Green and Bolognesi (1974) or sulfitolysis in 8 M guanidine hydrochloride (Edelman and Marchalonis, 1967), heavy and light polypeptide chains were obtained in a pure state. Lowry protein determinations (Lowry et al., 1951) showed that light chains had a higher absorbance at 280 nm than heavy chains. However, after correction of the difference in absorbance at 280 min and using molecular weights of 60.000 and 22,500 for H and L chains respectively, a ratio close to 1:1 was calculated.

The sedimentation equilibrium studies on heavy chains resulted in a molecular weight value comparable to those obtained for paddlefish (Acton et al., 1971b) and catfish (Hall et al., 1973; Mestecky et al., 1975). Using a direct way to determine the partial specific volume of heavy chains in the same solute as used for molecular weight determination, a value (0.736 ml/g) was found which is much higher than the value calculated from the amino acid and carbohydrate contents (0.714 ml/g). It is striking that Small and Lamb (1966) who measured partial specific volumes by another direct means (density gradient columns), also found higher values of $\bar{v}$ for heavy chains than for light chains or the intact immunoglobulin molecules.

The amino acid compositions of pike heavy and light chains presented in Table 2

were corrected for losses due to acid hydrolysis under our conditions: serine, threonine and valine by 10, 2 and 3% respectively (Kraal, 1972; Kraal et al., 1972). The composition of heavy and light chains is similar to those reported for other fish immunoglobulin chains (Acton et al., 1971a, 1971b, 1971c; Hall et al., 1973; Marchalonis and Edelman, 1966; Mestecky et al., 1975).

In conclusion it can be stated that the immunoglobulin of pike is structurally similar to the macroglobulins of other bony fishes (see Litman (1976) for a review). The data presented here are compatible with a tetrameric molecular form which has been demonstrated in the holostean fishes gar and paddlefish (Acton et al., 1971a, 1971b, 1971c) and the teleostean fishes bowfin, giant grouper, carp and catfish (Clem, 1971; Hall et al., 1973; Litman et al., 1971b; Mestecky et al., 1975; Shelton and Smith, 1970).

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