PHYLOGENY OF IMMUNOGLOBULIN STRUCTURE AND FUNCTION*

III. Immunoglobulins of the Chicken

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Fowl have been used in immunologic studies for more than 50 yr (1), but as yet little is known about the structure of their immunoglobulins. Based on the antigenicity of their heavy (H) chains (2), the domestic chicken, ring-necked pheasant (*Phasianus colchicus*), and Japanese quail (*Coturnix coturnix*) each contain at least two classes of immunoglobulins. Many reports in the literature tend to equate the chicken macroglobulin with IgM, and the low molecular weight molecule (~7S) is often equated with IgG of higher animals. These comparisons have been made without regard to the parameters necessary to designate classes.

In this paper, evidence is presented to show that the chicken 7S immunoglobulin varies in several important respects from mammalian IgG, whereas the macroglobulin is quite similar to mammalian IgM. In discussing these results, an attempt is made to better align chicken immunoglobulins with the immunoglobulins of mammals.

Materials and Methods

Purification of Chicken Macroglobulin.—A combination of successive Na_2SO_4 precipitations and recycling on 2.5 cm × 100 cm Sephadex G-200 columns was used as described previously (3), except that the buffer was 0.14 M NaCl in 0.015 M Tris-HCl, pH 7.4 (hereafter referred to as Sephadex buffer). Briefly, serum was precipitated three times with Na_2SO_4 (18%, 14%, 14% w/v). The final globulin-containing precipitate was dissolved in Sephadex buffer, dialyzed overnight at 4°C against Sephadex buffer, and precipitated with 9% w/v Na_2SO_4. The proteins thus precipitated were removed by centrifugation, and an additional 5% w/v Na_2SO_4 was added to the supernatant. The precipitate formed at this stage (5% precipitate) is designated as the 16.7S-rich fraction.

Purification of Chicken 7.1S Immunoglobulin.—Whole chicken serum was dialyzed against 0.015 M Tris-HCl pH 8.0, containing 1 M urea. The dialyzed serum (80 ml) was applied to a 4.5 cm × 50 cm DEAE(diethylaminoethyl)-cellulose column (DE-32 microgranular, Whatman) equilibrated with the same solvent. Elution was accomplished with a linear NaCl grad-

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dient. The starting buffer was 0.015 M Tris-HCl, pH 8.0 plus 1 M urea; the limit buffer was the starting buffer plus 0.6 M NaCl. The first major peak was concentrated by ultrafiltration and fractionated on Sephadex G-200 as described above.

Protein Concentration.—All column fractions were monitored for protein by absorbency at 280 mμ.

Radioiodination of Proteins.—Radioiodinations were done using the Chloramine T method (4).

Antigenic Analysis of Chicken Proteins.—Rabbit antisera to chicken serum and isolated polypeptide chains were prepared by emulsifying antigen solutions with equal volumes of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) and injecting the emulsion subcutaneously in multiple sites. Booster injections were given weekly, and the rabbits were bled 10–14 days after the third injection. The anti-whole chicken serum and anti-chicken globulin antisera used in the initial stage of this investigation were provided by Dr. Bruce Glick.

Ouchterlony analyses were performed in 1.5% Noble Agar (Difco) in barbital buffer, $\Gamma/2$ (ionic strength) = 0.15, pH 7.4 (5). Microimmunoelectrophoresis (6) was performed in 1% Agarose (Bausch and Lomb Inc., Rochester, N.Y.), using barbital acetate buffer, $\Gamma/2$ = 0.05, pH 8.6, on microscope slides. Approximately 220 v were applied for 60 min.

Preparation of Polypeptide Chains.—Extensive reduction with 0.1 M dithioerythritol (Cyclo Chemical Corp., Los Angeles, Calif.) and alkylation of chicken immunoglobulins in 7 M guanidine-HCl, followed by separation of the resultant polypeptide chains by gel filtration through an upward flow Sephadex G-200 column equilibrated with 5 M guanidine-HCl, was performed as described previously (7). The column dimensions were 120 cm × 2.5 cm. Partial reduction and alkylation was performed in Sephadex buffer, using the same concentrations of reducing and alkylating agents as used for the extensive reductions. Protein solutions were buffered to pH 8.0 by the addition of 0.25 volumes of 4.6 M Tris-HCl and reduced at room temperature for 1 hr. After chilling in an ice bath, alkylation for 1 hr was accomplished with iodoacetamide. The reduced and alkylated proteins were dialyzed for 24 hr in the cold against Sephadex buffer, made up to 5.6 M by the addition of guanidine-HCl, and applied to Sephadex G-200 columns equilibrated with 5 M guanidine-HCl (8).

Analytical Ultracentrifugation.—Sedimentation velocity studies were performed in the Spinco Model E ultracentrifuge. Molecular weights were determined by the high speed sedimentation equilibrium method of Yphantis (9), utilizing interference optics and a RTIC temperature control unit. Fringe patterns were recorded on Kodak II-G spectrographic plates. Measurements of fringe patterns against radial distance were made with a Nikon comparator.

Molecular weights were calculated, using the appropriate density and assuming a partial specific volume ($\bar{v}$) of 0.71.

Solvent densities for solutions containing mixtures of urea, iodoacetamide, and Sephadex buffer were determined pycnometrically.

Determination of Extinction Coefficients.—Extinction coefficients (1 cm and 1% at 280 mμ) were calculated for the purified proteins as follows: Concentrated solutions of protein were exhaustively dialyzed against 0.3 M KCl and then divided into portions; some of these were then dried to constant dry weight, and others were diluted in either 0.3 M KCl, 0.1 M NaOH, or 5 M guanidine-HCl, and read for ultraviolet (280 mμ) absorbency in a Zeiss model PMQ II spectrophotometer. The dry weight of an equal volume of the 0.3 M KCl dialysate was subtracted from the dry weight of the protein-KCl mixture.

Hexose Determination.—Protein-bound hexose was measured by the orcinol method of Winzler (10), using a galactose-mannose (1:1) standard.

Control Proteins.—Human colostral IgA was a gift from Jean Curry. Rabbit gammaglobulins were obtained from Pentex Inc., Kankakee, Ill.; bovine serum albumin (BSA) was ob-

tained from Armour Pharmaceutical Co., Kankakee, Ill.; and the human IgG was supplied by Dr. R. T. Smith. Nurse shark 7S immunoglobulin was prepared as described previously (11).

RESULTS

Isolation of Chicken Immunoglobulins.—Chicken serum contains several components which precipitate when dialyzed against 0.015 M Tris-HCl buffer,

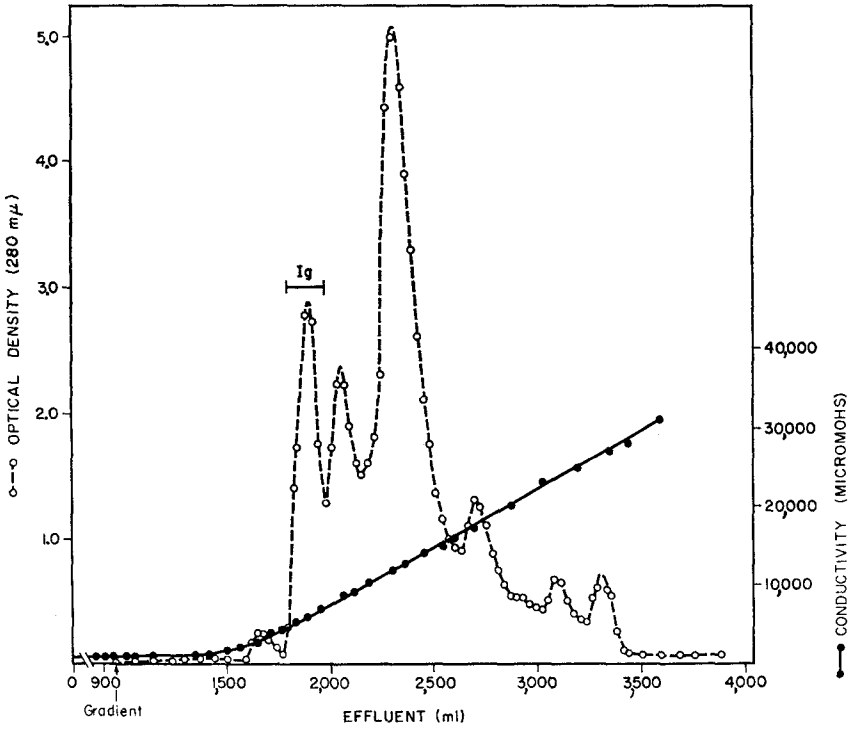

FIG. 1. Ion exchange chromatography of whole chicken serum on DEAE-cellulose

pH 8.0. The addition of 1 M urea to this buffer prevented precipitation and thus allowed chromatography of the whole serum. Fig. 1 shows the elution profile obtained using a DEAE-cellulose column equilibrated with 0.015 M Tris-HCl plus 1 M urea. The first peak obtained after the salt gradient has been initiated (labeled Ig for immunoglobulin on the basis of its reaction with a rabbit anti-chicken γ-globulin antiserum provided by Dr. Bruce Glick, and also because it contained a protein antigenically identical to a chicken immunoglobulin prepared in an entirely different manner—see reference 12) was concentrated by postiive pressure, dialyzed against Sephadex buffer, and subjected to gel filtra-

tion on Sephadex G-200. This procedure resulted in two fractions (Fig. 2), the heavier of which contained only 7.1S immunoglobulin and represented ~2 mg immunoglobulin per ml of serum. The second fraction from the Sephadex G-200 column was pink. This material will subsequently be referred to as the pink protein. In contrast to most mammalian sera, very little protein was eluted with the initial low ionic strength buffer.

Fig. 3 shows the Sephadex G-200 elution profile of the 5% Na_2SO_4 (16.7S-rich) precipitate. The earliest eluting fraction, when recycled through Sephadex G-200, eluted as a single symmetrical peak. In this manner, approximately 0.3–

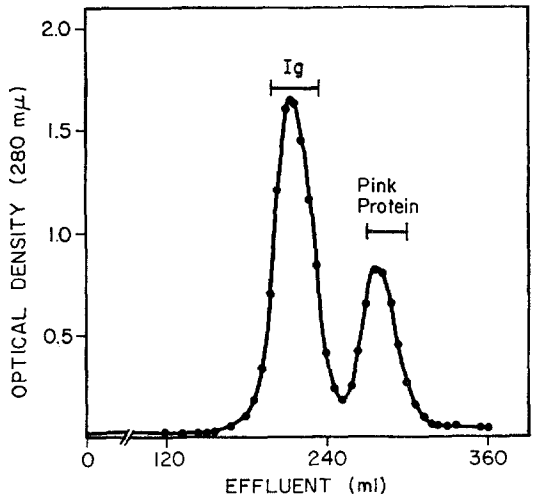

FIG. 2. Gel filtration on Sephadex G-200 of the Ig fraction obtained from the DEAE-cellulose column.

0.4 mg of macroglobulin could be obtained per ml of starting serum. A previous study has shown that this method does indeed result in the isolation of macroglobulin antibody (3). Fig. 9a shows that these isolation procedures resulted in the purification of two immunoglobulins that are antigenically pure and distinct from one another. The pink protein (Fig. 9c) gave a reaction of nonidentity with 7.1S immunoglobulin, albumin, and L chains. Immunoelectrophoretic patterns of the two immunoglobulins, pink protein and albumin, are shown in Fig. 4.

Sedimentation Coefficients.—Whole chicken serum contained ~31S, 16.1S, 7.3S, and 4.0S components (Fig. 5a, b) when studied by sedimentation velocity. The sedimentation coefficient of the purified low molecular weight immunoglobulin was 7.1S at ~7 mg/ml (Fig. 5c). The macroglobulin fraction contained both 16.7S and 28S components (Fig. 5d).

The presence of a large amount (up to 40%) of a ~28S component in the

purified macroglobulin preparation and a very small proportion of a similar component in whole serum suggested that considerable aggregation had oc-

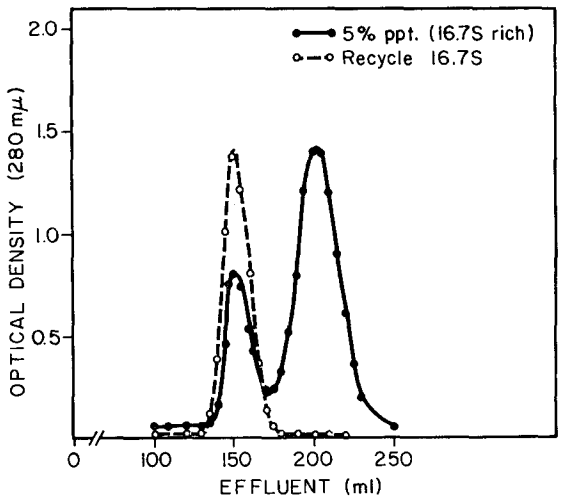

FIG. 3. Gel filtration on Sephadex G-200 of 5% sodium sulfate precipitated globulins and recycle of the excluded protein.

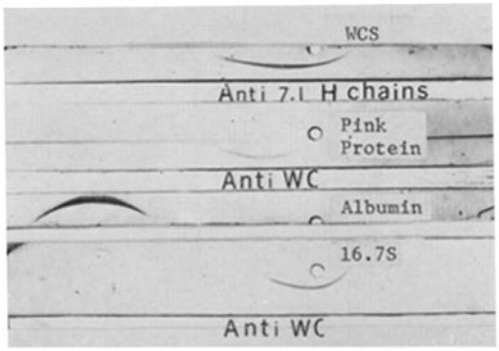

FIG. 4. Immunoelectrophoretic analysis of whole chicken serum (WCS), pink protein, albumin, and 16.7S immunoglobulin. The antisera were rabbit anti 7.1S H chains and rabbit anti-whole chicken serum (anti-WC).

curred during the isolation procedures and that these two boundaries did not represent two different proteins. To test this hypothesis, a portion of the purified macroglobulin was made up in 3 M urea (Fig. 5e), and another portion was made 5 M with respect to guanidine-HCl. In both cases, most of the 28S material was converted to the monomeric form, which supported the concept of non-

covalent aggregation. However, some heavy component remained,and a slowly sedimenting specie was generated in the presence of urea. To test for disulfide interchange as a mechanism for generating the "low molecular weight component", 0.01 M iodoacetamide was added to the protein solution prior to the addition of 3 M urea. No new "slowly sedimenting specie" was formed under these

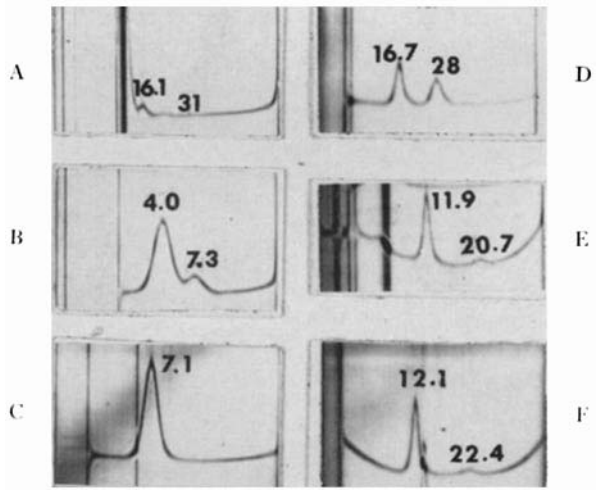

FIG. 5. Schlieren patterns and sedimentation coefficients of chicken proteins:
A. Whole chicken serum (diluted 1:3) after 1 min.
B. Same as A except after 40 min.
C. Light immunoglobulin (~7 mg/ml).
D. Macroglobulin (~4.5 mg/ml).
The solvent for A–D was Sephadex buffer. The numbers above the boundaries represent S_{240w}.
E. Macroglobulin (~4.5 mg/ml) in Sephadex buffer containing 3 M urea.
F. Macroglobulin (~4.5 mg/ml) in Sephadex buffer containing 3 M urea and 0.01 M iodoacetamide.
The numbers represent S_{obs}.

conditions (Fig. 5f). Thus, it would seem that the iodoacetamide prevented disulfide interchange.

Extinction Coefficients and Hexose Contents.—The extinction coefficients and hexose contents of chicken 16.7S and 7.1S immunoglobulins are given in Table I. The extinction coefficients of bovine serum albumin (BSA) and rabbit IgG, and the hexose content of rabbit IgG, nurse shark 7S immunoglobulin, and human IgG are included for control purposes.

A very similar hexose content was found for the two chicken immunoglobulins. These values were two to three times higher than the values obtained for human and rabbit IgG and about one-half of that obtained for nurse shark 7S IgM.

Molecular Weights of Chicken 16.7S and 7.1S Immunoglobulins.—The molecular weights of the 16.7S and 7.1S immunoglobulins are presented in Table II, and representative plots of the logarithm (Ln) of the concentration of protein as a function of the radius squared (Fig. 6) indicated that over the measurable

TABLE I

Extinction Coefficients and Hexose Content of Chicken Immunoglobulins

Proteins	Solvent	Extinction coefficient	Hexose content
		$\epsilon_{280\ m\mu,\ 1\%}^{1\ cm} \pm$ SD*	% ±SD
Chicken 16.7S	0.3 M KCl	12.72 ± 0.77	2.60 ± 00
	0.1 N NaOH	12.52 ± 0.76	
	5 M guanidine	11.11 ± 0.68	
Chicken 7.1S	0.3 M KCl	13.18 ± 0.04	2.22 ± 0.22
	0.1 N NaOH	14.42 ± 0.04	
	5 M guanidine	12.74 ± 0.19	
Rabbit IgG	0.3 M KCl	15.17 ± 0.19	0.79 ± 0.13
	0.1 N NaOH	16.75 ± 0.11	
	5 M guanidine	14.99 ± 0.04	
BSA	0.3 M KCl	7.44 ± 0.21	
	0.1 N NaOH	8.68 ± 0.22	
	5 M guanidine	6.90 ± 0.19	
Nurse Shark 7S Ig			4.16 ± 0.76
Human IgG			0.98

* SD, standard deviation.

TABLE II

Weight Average Molecular Weights × 10^3 of Chicken Immunoglobulins

Protein	rpm	Initial concentration			Average ± SD*
		0.25 mg/ml	0.5 mg/ml	0.75 mg/ml	
Chicken 16.7S‡	8,225	857	813	800	823 ± 30
§	8,225	987	904	972	954 ± 139
Chicken 7.1S‖	13,410	175	180	156	170 ± 12

* SD, standard deviation.

‡ Solvent was 3 M urea + 0.01 M iodoacetamide + Sephadex buffer, $\rho = 1.0512$.

§ Solvent was ~1.9 M urea + 0.01 M iodoacetamide + Sephadex buffer, $\rho = 1.0323$.

‖ Solvent was Sephadex buffer, $\rho = 1.0062$.

range, the heterogeneity was minimal. It must be emphasized, however, that in the case of the macroglobulin preparations, the ~28S component was sedimented to the bottom of the cell, and thereby limited measurements in this area.

The molecular weight of the 16.7S immunoglobulin in the two different urea-containing solvents varied somewhat. By averaging the values obtained at the three different protein concentrations, a mean value of 823,000 was obtained in

3 M urea and 954,000 in the 1.9 M urea solvent. An average of these two values gives ~890,000.

The molecular weight of the 7.1S immunoglobulin appeared to have slight, if any, concentration dependence. The mean value, over the three protein concentrations employed, was about 170,000.

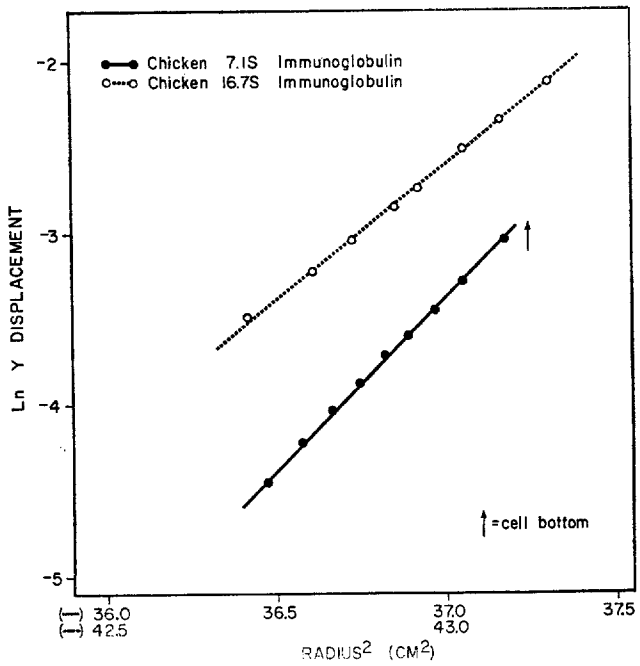

FIG. 6. Plots of the logarithm of the concentration (in vertical displacement from the baseline of a fringe in cm) versus distance from the center of rotation squared for chicken 16.7S and 7.1S immunoglobulins. The solvent for the 7.1S immunoglobulin was Sephadex buffer. Sephadex buffer containing 3 M urea and 0.01 M iodoacetamide was used for the 16.7S immunoglobulin. Centrifugation was performed at 20°C for 36 hr; a speed of 13,410 rpm was used for the 7.1S immunoglobulin while 8225 was used for the 16.7S immunoglobulin.

Polypeptide Chains from Chicken Immunoglobulins: Fractionation and Molecular Weights.—The molecular weights of the component polypeptide chains of the chicken 16.7S and 7.1S immunoglobulins were estimated by filtration of the extensively reduced and alkylated proteins through columns of Sephadex G-200 equilibrated with 5 M guanidine-HCl. The unreduced proteins were also filtered in the same column. No dissociation was detected with either the unreduced 16.7S or the 7.1S protein.

Each time a determination was to be made, an internal marker of ^{125}I-labeled immunoglobulin (immunoglobulins whose heavy (H) and light (L) chain molec-

ular weights had previously been calculated were used) was added prior to the reduction and alkylation. Only a few micrograms of labeled protein standards were necessary and thus did not contribute significantly to the optical density readings.

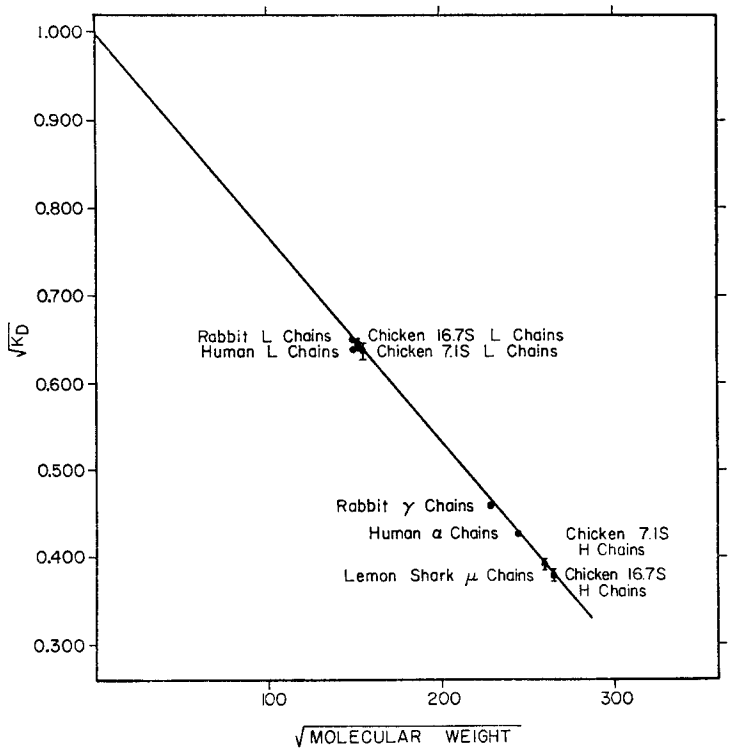

FIG. 7. Plot of $\sqrt{K_D}$ vs $\sqrt{\text{mol wt}}$ for extensively reduced and alkylated materials filtered through a Sephadex G-200 column equilibrated with 5 M guanidine-HCl. The vertical bars represent the range of K_D's obtained. ($K_D = V_E - V_O/V_i$ where V_E is the peak elution volume, V_O, excluded volume, and V_i, the included volume. $V_O + V_i$ = the elution volume for small molecules).

Assuming that the extinction coefficients of the H and L chains of the two chicken immunoglobulins are approximately equal, it was calculated, based upon optical density (280 mμ) recoveries, that for each molecule, the H chains account for ~75% of the mass, and the L chains make up the remaining 25%.

The configuration of the H and L chains prepared by reduction and alkylation in 5 M guanidine-HCl should be that of random coils. Thus, by minimizing the shape factors, the elution position of the polypeptide of unknown molecular weight relative to the radiolabeled internal markers gives a good measurement

of mass. A plot of the square root of K_D vs the square root of molecular weight, as suggested by Andrews (13) and used recently by Clem and Small (14), with markers of known molecular weights (Fig. 7) shows that the L chains of chicken

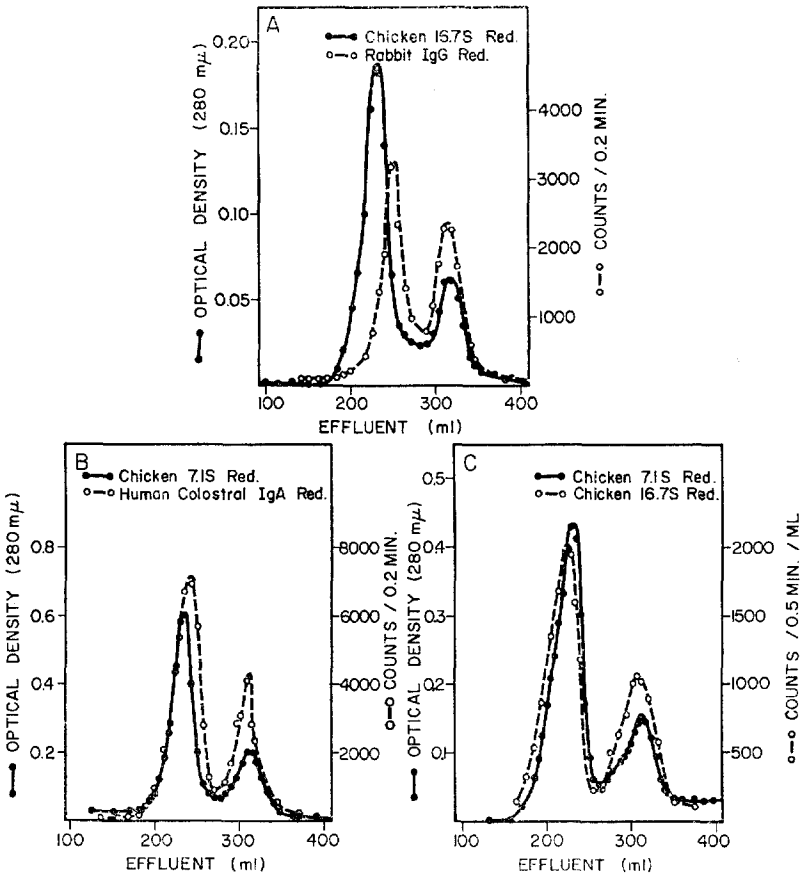

FIG. 8. Sephadex G-200 gel filtration in 5 M guanidine-HCl of extensively reduced and aklylated (Red.) immunoglobulins. The rabbit IgG, human colostral IgA, and chicken 16.7S immunoglobulin used in A, B, and C were each radiolabeled with ^{125}I.

7.1S and 16.7S immunoglobulins are very similar to the L chains from human and rabbit IgG, thereby indicating a mol wt of about 22,000. The H chains from the 16.7S immunoglobulin eluted in a position corresponding to a mol wt of 70,000. A typical elution profile for reduced 16.7S immunoglobulin is shown in Fig. 8a. The H chains from the 7.1S immunoglobulin were slightly smaller than those from the chicken 16.7S and the lemon shark 19S molecules. They eluted in

a position slightly ahead of the H chains of human colostral IgA (Fig. 8b) and slightly later than the H chains of chicken 16.7S (Fig. 8c). This indicated a mol wt of approximately 67,000 for the H chains from the 7.1S immunoglobulin.

For the purpose of isolating antigenically reactive H and L chains, the same guanidine-HCl columns were employed. Although the separation of the polypeptide chains was not as distinct as with the extensively reduced proteins (due to probable H–H or H–L aggregates), antigenically pure chains were isolated by recycling on this column.

Antigenic Analyses.—The two chicken immunoglobulins exhibited a reaction of nonidentity (Fig. 9a), as did their isolated H chains (Fig. 9b) when examined

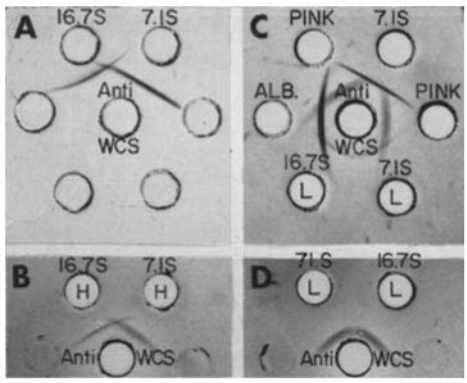

FIG. 9. Ouchterlony analysis of purified chicken serum proteins. Anti-WCS, rabbit antiserum to whole chicken serum; 16.7S and 7.1S are the isolated immunoglobulins; alb., albumin; pink, pink protein isolated by DEAE-cellulose and Sephadex G-200; L and H refer to light and heavy chains, respectively. The concentration of each protein was approximately 2 mg/ml.

with rabbit antisera prepared against whole chicken serum. Antisera prepared against the isolated H chains of one class of immunoglobulin did not react with the H chain of the other class. Rabbit antisera to 7.1S L chains and to whole chicken serum (Fig. 9d) gave reactions of identity with isolated L chains from the 16.7S and 7.1S molecules.

DISCUSSION

As has been pointed out in previous studies, chickens, pheasants, and Japanese quail (2) each possess at least two distinct classes of immunoglobulins based upon antigenic differences in the H chains. With very little physical-chemical data to rely upon, these classes were designated as IgM and IgG in an attempt to relate them to mammalian immunoglobulins. Recent evidence presented by Mehta and Tomasi[1] employing complement fixation techniques

[1] Personal communication.

indicated that the chicken macroglobulin cross-reacts with human IgM, whereas the chicken 7S molecule does not cross-react with human IgG. The main purpose of this discussion will be to point out that, on one hand, the chicken 16.7S molecule does resemble human and rabbit IgM in terms of gross architecture and that, on the other hand, the chicken 7.1S immunoglobulin does not appear to resemble any described mammalian immunoglobulin.

The method we employed in preparing chicken 7.1S immunoglobulin has not been used in this system previously, and thus we may have been working with a different immunoglobulin than that used by other investigators. This seems highly unlikely, since the antisera prepared against the H chains of this protein react (identity) with the H chains of the ~7S molecule prepared by several other methods. This in itself does not rule out the existence of other classes of immunoglobulins or subclasses of the 7.1S and 16.7S immunoglobulins. In fact, the 7.1S immunoglobulin appears to consist of at least two populations, based on digestibility with pepsin (2).

The 16.7S chicken immunoglobulin has a mol wt of about 900,000. The H chains and L chains account for 75% and 25%, respectively, of the mass of the molecule, and their mol wt are ~70,000 (H chains) and ~22,000 (L chains). Using the polypeptide chain molecular weight data, the H-to-L-chain mass ratio, and assuming a 2H–2L chain subunit structure, the subunit would be expected to have a mol wt of about 180,000. Furthermore, on the basis of a pentameric structure, a mol wt of ~900,000 would be predicted. The observed mol wt of ~890,000 is in excellent agreement with the expected value. However, preliminary experiments designed to prepare and calculate the molecular weight of the chicken 16.7S subunit have so far met with only limited success. The major reductive subunit has a mol wt of ~174,000.[2] However, unlike the IgM of other species, chicken 16.7S macroglobulin appears to dissociate into lighter fragments (120,000–150,000) under certain conditions of reduction. Release of L chain antigenic determinants in neutral aqueous buffers from the reduced macroglobulin of the pheasant (2) and chicken (3, 15) has previously been observed, and thus may account for the variable molecular weight subunits.

The hexose content of the chicken 16.7S immunoglobulin (2.6%) is much higher than that of rabbit and human IgG (<1%) but lower than the ~5% observed with human IgM (16). In the absence of total carbohydrate measurements on this molecule, it is difficult to decide on the significance of this apparent difference.

Considerable attention has been focused on the chicken 7.1S immunoglobulin in the past, due to several unique properties. It associates in 1.5 M NaCl to give a 14S aggregate and may account for the "high-salt" phenomenon with chicken immune precipitates (17, 19). Furthermore, it releases light chains under certain conditions of partial reduction (18); mammalian immunoglobulins do not.

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[2] Leslie and Clem. Unpublished observations.

Mol wts of 150,000 (19), 206,000 (20), as well as some intermediate values, have been reported (21,22). Our sedimentation equilibrium data suggest that the molecular weight of the intact 7.1S immunoglobulin has little, if any, concentration dependence; and an average obtained with the three concentrations employed yielded a mol wt of ~170,000. The mol wt of the H chains (75%) and L chains (25%) were calculated to be ~67,500 and 22,000, respectively. Thus, a four-chain structure (2H and 2L) would have a mol wt of ~179,000, which is in good agreement with the value we obtained. Furthermore, this value is consistent with that reported recently for chicken antibody to DNP (22). This value is higher than that reported for all studied mammalian IgG and IgA molecules. It is quite similar to the ~183,000 recently reported for human IgD (23). It is lower than the ~900,000 obtained for pentameric IgM and the 200,000 obtained for a human IgE molecule.

However, it has now become obvious that one cannot assign immunoglobulin class distinctions based only upon molecular weights of intact immunoglobulin molecules. For example, sharks have both 19S and 7S IgM molecules (14). Similarly, human cord serum (24), as well as serum obtained from patients with certain dyscrasias of the lymphoid system (25), contains molecules which behave as 7S IgM. The presence of what appears to be both 7S and 19S IgG in the serum of newborn piglets (26) would also serve as an example here. However, it appears that in most cases the properties of the immunoglobulin H chain are the important factors in making class designations; in fact, one could argue that amino acid sequences would give the ultimate comparative answer. In the absence of sequence data, one must proceed with the usage of "grosser" parameters such as polypeptide chain molecular weights and electrophoretic mobilities. The above mentioned studies with shark immunoglobulins show these molecules to be at least IgM-like on the basis of several H chain properties. Similarly, the two frog immunoglobulin classes have been designated as IgM and IgG due to the similarity of their H chains to the two respective mammalian γ and μ chains. Therefore, if one considers the chicken 7.1S H chain in light of such parameters, it appears not to have a mammalian counterpart. That is to say that the chicken 7.1S H chain mol wt of 67,500 is somewhat too small to be a μ chain (mol wt ~70,000) or an ϵ chain (mol wt ~80,000). Similarly, it is too large to be a γ (mol wt ~50,000) or an α (mol wt~60,000). The molecular weight of human δ chains have not yet been determined. It is difficult to decide on the significance of H chain electrophoretic mobility in this respect, but it should be pointed out that Mehta and Tomasi (27) have reported that while the chicken macroglobulin H chain is μ-like, the 7.1S H chain is considerably different from mammalian γ chains.

A hexose content of 2.2% for the 7.1S molecule is similar to that reported by Tenenhouse and Deutsch (20) for chickens, similar to chicken 16.7S, higher than mammalian IgG, and lower than the values reported for human IgA, IgD, and IgM.

Thus, in conclusion, chicken macroglobulin resembles mammalian IgM in gross architecture, whereas the 7.1S immunoglobulin does not really align itself with IgG, IgA, IgD, IgE, or IgM. This matter may be resolved when amino acid sequence data become available. We propose the usage of IgM and IgY for the macroglobulin and 7.1S molecules, respectively. For those interested in a more extensive discussion of the phylogeny of immunoglobulin structure and function, a new review is suggested (28).

SUMMARY

Chicken 7.1S immunoglobulin was purified from whole chicken serum by DEAE-cellulose chromatography and Sephadex G-200 gel filtration. The macroglobulin was purified by a combination of salt precipitation and Sephadex G-200 gel filtration. Both immunoglobulin molecules yielded 75% heavy (H) chains and 25% light (L) chains when subjected to extensive reduction and alkylation followed by gel filtration in 5 M guanidine-HCl. Antigenically reactive H and L chains were obtained by partial reduction and alkylation followed by gel filtration in 5 M guanidine-HCl. The 7.1S and 16.7S immunoglobulin H chains were antigenically unrelated to each other, whereas the L chains were antigenically indistinguishable from one another. The 16.7S H chains were found to have a mass of ~70,000, and the 7.1S H chains had a mass of 67,500. The mass of the L chains was ~22,000.

Sedimentation equilibrium studies of the 7.1S immunoglobulin molecule gave a mol wt of ~170,000 which is in good agreement with the 179,000 predicted on the basis of 2 H and 2 L polypeptide chains. The 16.7S molecule was shown to have a mol wt of ~890,000. A reductive subunit that has a mol wt of ~174,000 has been isolated from the 16.7S molecule. These values are consistent with the chicken macroglobulin having five subunits, each of which has 2 H and 2 L chains. The hexose contents of the chicken 7.1S and 16.7S immunoglobulins are 2.2% and 2.6%, respectively. The extinction coefficients of the 7.1S and 16.7S immunoglobulins were 13.18 ± 0.04 and 12.72 ± 0.77, respectively, when measured in 0.3 M KCl.

Based upon physical-chemical and antigenic characteristics, the 16.7S immunoglobulin most closely resembles IgM of mammals. The 7.1S immunoglobulin definitely belongs to a different class than the 16.7S immunoglobulin, but it does not align itself very well with any of the mammalian immunoglobulins. We propose that this molecule be designated as IgY. Furthermore, this designation would be useful for the immunoglobulins of other species for which there is insufficient correlation with any of the known human immunoglobulins.

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