Chemical Properties of Guinea-Pig Immunoglobulins γ_1 G, γ_2 G and γ M

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(Received 27 July 1970)

The isolation of guinea-pig immunoglobulins γ_1 G, γ_2 G and γ M are described and methods for separating the polypeptide chains of each examined. The molecular weights, extinction coefficients and carbohydrate and amino acid compositions of the immunoglobulins and their constituent chains have been analysed. The findings provide a basis for further studies attempting to relate structural differences to distinct biological properties of guinea-pig immunoglobulins.

Guinea-pig serum contains two immunoglobulins referred to as immunoglobulins γ_1 G and γ_2 G (Yagi, Maier & Pressman, 1962; Benacerraf, Ovary, Bloch & Franklin, 1963; White, Jenkins & Wilkinson, 1963) with sedimentation coefficients of 7S and similar hexose contents. The two proteins have distinct immunological and biological properties, and anti-dinitrophenol antibodies belonging to immunoglobulins γ_1 G and γ_2 G are known to differ in the amino acid composition of their heavy chains (Lamm, 1969).

An attempt is being made in this laboratory to relate structural differences between guinea-pig immunoglobulin molecules to their specific biological properties. As a basis for this investigation, immunoglobulins γ_1 G, γ_2 G and γ M have been isolated from guinea-pig serum without recourse to purification based on combining specificity. Some physicochemical properties of these immunoglobulins and their constituent polypeptide chains are reported in this paper.

MATERIALS AND METHODS

Animals

Outbred guinea pigs (300–500 g) and rabbits (2–3 kg) were used.

Isolation of immunoglobulins

Preliminary immunization of some guinea-pig blood donors was carried out to boost concentrations of specific and non-specific immunoglobulin (Binaghi, 1966; see Table 6). Animals were given 0.5–1.0 mg of bovine γ -globulin in Freund's adjuvant (complete or incomplete) by intramuscular injection on days 1 and 8 followed by intraperitoneal injection of alumina-adsorbed bovine γ -globulin (about 2 mg) in suspension in 0.9% NaCl on day

28; animals were bled by cardiac puncture on days 35 and 42 and pooled sera stored at -15°C for up to 6 months.

Preparation of immunoglobulin γ M. Pooled sera from normal or immune animals were centrifuged at 15000 rev./min at 2°C and dialysed for 18–20 h at 2°C against 30 vol. of 2 mM-sodium phosphate buffer, pH 6.3. The euglobulin precipitate was washed three times at 2°C with the same buffer and dissolved in 0.1 M-tris-HCl–0.2 M-NaCl buffer, pH 8.2. A solution of aqueous NaCl (153 g/l) and KBr (354 g/l) having a density of 1.346 g/ml was added to the euglobulin solution to raise the specific gravity to 1.065 and ultracentrifugation was performed at 100000 g for 18 h at 10°C . The viscous protein layer occupying the bottom third of the tubes was dialysed against 0.05 M-tris-HCl–0.1 M-NaCl buffer, pH 8.2, and eluted with the same buffer from a Sephadex G-200 column (140 cm $\times$ 3.3 cm). The peak eluted with the void volume contained immunoglobulin γ M and was concentrated by pressure dialysis against the above tris buffer. This fraction was stored at 2°C since freezing or freeze-drying led to considerable denaturation of immunoglobulin γ M.

Preparation of immunoglobulins γ_1 G and γ_2 G. Since immunoglobulin γ_1 G is present in serum in relatively low concentration it is difficult to isolate it free of immunoglobulin γ_2 G. A preliminary study of the relative precipitability of immunoglobulins γ_1 G and γ_2 G in $(\text{NH}_4)_2\text{SO}_4$ solutions was therefore carried out in an attempt to prepare enriched immunoglobulin γ_1 G solutions. For this purpose pure preparations labelled with ^{131}I and ^{125}I were used. The immunoglobulin γ_2 G was prepared from guinea-pig anti-(bovine γ -globulin) serum by precipitation in 40% saturated $(\text{NH}_4)_2\text{SO}_4$ followed by elution from DEAE-cellulose (Whatman DE22) with 0.1 M-tris-HCl buffer, pH 8.2. The eluted immunoglobulin γ_2 G fraction, which gave a single arc on immunoelectrophoresis against rabbit anti-guinea-pig serum, was labelled with ^{131}I (McFarlane, 1958). Gradient elution of the same DEAE-cellulose column with a second buffer (0.1 M-tris-HCl–0.4 M-NaCl buffer, pH 8.2) gave a fraction containing mainly immunoglobulin γ_1 G together with a trace of immunoglobulin γ_2 G and some non-immunoglobulin protein. This fraction was adsorbed to cellulose-bound bovine γ -globulin (Robbins, Haimorich & Sela, 1967) and the antibody to bovine γ -globulin eluted with 0.1 M-acetic

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Fig. 1. Ammonium sulphate precipitation of radioactive iodine-labelled guinea-pig immunoglobulins γ_1 G ($\blacktriangle$), γ_2 G prepared as described in the Materials and Methods section ($\bullet$), γ_2 G (Prep. B) prepared by chromatography of whole serum on DEAE-cellulose without preliminary $(\text{NH}_4)_2\text{SO}_4$ precipitation ($\circ$) and γ M ($\triangle$).

acid. The eluted protein after neutralization to pH 7 gave only an immunoglobulin γ_1 G arc on immunoelectrophoresis and was labelled with ^{125}I . The ^{131}I -labelled γ_2 G and ^{125}I -labelled γ_1 G immunoglobulins were added to guinea-pig serum, the total volume of labelled immunoglobulins being less than 4% of the volume of carrier serum. Samples (1 ml) were mixed with $(\text{NH}_4)_2\text{SO}_4$ saturated at 22°C and with 0.9% NaCl in appropriate proportions to give a range of $(\text{NH}_4)_2\text{SO}_4$ concentrations (24–50% saturation) in a final volume of 2 ml. After 1 h at 22°C the proportions of immunoglobulins γ_1 G and γ_2 G precipitated were determined by scintillation counting. As shown in Fig. 1, which includes data for immunoglobulin γ M, the immunoglobulin γ_1 G fraction was preferentially precipitated at low $(\text{NH}_4)_2\text{SO}_4$ concentration. Accordingly, serum (or euglobulin supernatants) from animals immunized with bovine γ -globulin was precipitated three times in 32%-saturated $(\text{NH}_4)_2\text{SO}_4$ and the final precipitate, after dialysis against 0.1 M-tris-HCl buffer, pH 8.2, was fractionated on DEAE-cellulose. Immunoglobulin γ_2 G was eluted with 0.1 M-tris-HCl buffer, pH 8.2, and the column was washed with 0.1 M-tris-HCl-0.02 M-NaCl buffer to elute the remaining immunoglobulin γ_2 G. A gradient to 0.1 M-tris-HCl-0.4 M-NaCl buffer, pH 8.2, was then used to elute immunoglobulin γ_1 G (Fig. 2). Additional immunoglobulin γ_2 G was obtained by a further salt precipitation of the serum supernatant at 40%-saturated $(\text{NH}_4)_2\text{SO}_4$ followed by similar anion-exchange fractionation. Both immunoglobulin γ_1 G and immunoglobulin γ_2 G were finally subjected to gel filtration on Sephadex G-200 in 0.05 M-tris-HCl-0.1 M-NaCl buffer, pH 8.2, to remove any immunoglobulin fragments that may have been present as a result of proteolysis occurring in the original serum.

Preparation of immunoglobulin γ M subunits. Immunoglobulin γ M was reduced for 8 min at 25°C with 0.05 M-cysteine in 0.2 M-tris-HCl buffer, pH 8.6 (Miller & Metzger, 1965); the mixture was cooled to 0°C and a 10% molar excess of iodoacetamide in the tris-HCl buffer was added.

Fig. 2. DEAE-cellulose chromatography of 32%-saturated- $(\text{NH}_4)_2\text{SO}_4$ precipitate of guinea-pig serum. Initial buffer, 0.1 M-tris-HCl, pH 8.2. Second buffer, 0.1 M-tris-HCl-0.02 M-NaCl, pH 8.2. Gradient, 0.02–0.4 M-NaCl in 0.1 M-tris-HCl buffer, pH 8.2, with constant-volume mixing chamber (500 ml). Flow rate, 30 ml/h. Fraction volume, 5 ml. Break in pre-gradient elution curve indicates start of second buffer. Arrows indicate the fractions pooled. Initial volume of serum, 130 ml. Protein on column, about 300 mg. Wt. of DEAE-cellulose, 30 g.

The reduced immunoglobulin γ M was dialysed against 0.05 M-tris-HCl-0.1 M-NaCl buffer, pH 8.2, and the monomer subunit isolated on a column (3 cm $\times$ 140 cm) of Sephadex G-200.

Preparation of immunoglobulin chains. Immunoglobulin preparations were reduced with 0.3 M- β -mercaptoethanol in 0.5 M-tris-HCl buffer, pH 8.0 (Fleischman, Porter & Press, 1963), or in 6 M-guanidinium chloride (Small & Lamm, 1966), alkylated with iodoacetamide and fractionated by filtration through Sephadex G-200 in 0.1 M-formic acid–1.0 M-acetic acid or 5 M-guanidinium chloride.

Analytical methods

Carbohydrate analyses. Proteins for analysis were precipitated with 50%-saturated $(\text{NH}_4)_2\text{SO}_4$ and dialysed against 0.9% NaCl. Hexose was determined by the method of Winzler (1955) with a 1:1 galactose:mannose standard; hexosamine (Johansen, Marshall & Neuberger, 1960), fucose (Dische & Shettles, 1948) and sialic acid (Aminoff, 1961) were measured by the described techniques.

Amino acid analyses. These were performed on a Locarte analyser by the method of Moore, Spackman & Stein (1958). Duplicate samples containing 1 mg of protein and 0.2–0.4 μmol of norleucine standard were hydrolysed in 6 M-HCl for 24 and 72 h in sealed evacuated tubes at 110°C (Crumpton & Wilkinson, 1963). Two analyses were performed on each sample by both the standard two-column system and a single-column three-buffer system (Hamilton, 1958). Buffers, elution times and temperatures were modified to suit the Locarte resins. Cysteine was determined as *S*-carboxymethylcysteine and tryptophan by the method of Melamed & Green (1963).

Specific extinction coefficients. These were determined

by using solutions of whole immunoglobulins in 0.5% $(\text{NH}_4)_2\text{CO}_3$ and isolated chains in 0.1M-acetic acid. Concentrations of the solutions were measured by precipitating protein with 40%-saturated $(\text{NH}_4)_2\text{SO}_4$ to eliminate soluble dextrans; salt was removed by dialysis against several changes of 0.5% $(\text{NH}_4)_2\text{CO}_3$ or 0.1M-acetic acid and samples were dried to constant weight at 100°C in a drying pistol containing P_2O_5 .

Molecular weights. These were determined for the whole immunoglobulins and γM subunits from sedimentation coefficients measured in a Spinco model E ultracentrifuge at three concentrations of each protein in 0.1M-HCl-0.05M-tris-HCl buffer, pH 7.95 at 20°C. The s_{20} values were extrapolated to zero concentration and the limiting sedimentation coefficients (s_{20}^0) were converted into the reference state of water at 20°C ($s_{20,w}^0$). Molecular weights were estimated from the partial specific volumes and diffusion coefficients calculated for human immunoglobulins by Miller & Metzger (1965). Molecular weights of the constituent heavy and light chains were estimated by gel filtration in triplicate of the totally reduced polypeptides through Sephadex G-200 (2.5 cm $\times$ 100 cm column adapted for upward flow) in 5M-guanidinium chloride (Cebra & Small, 1967). The void volume of the column was estimated by using human immunoglobulin γM , and totally reduced samples of rabbit immunoglobulin γG , bovine serum albumin and ^{131}I -labelled ribonuclease were used as standards.

RESULTS

Purity and yields of immunoglobulins

The yield of immunoglobulin γM from guinea pig serum immunized with bovine γ -globulin was 10–35mg/100ml of serum. Attempts to prepare immunoglobulin γM from the serum of unimmunized animals gave very much lower yields. On

immunoelectrophoresis (Scheidegger, 1955) with a polyvalent serum against guinea-pig serum raised in rabbits, the immunoglobulin γM preparations showed a single arc characteristically close to the origin (Fig. 3) and occasionally an ill-defined precipitate probably attributable to lipoprotein contamination. The purity of these preparations, as judged by the recovery of heavy and light chains after gel filtration of totally reduced immunoglobulin γM , on Sephadex G-200 in 5M-guanidinium chloride, was 88–96%.

The yield of immunoglobulin $\gamma_2\text{G}$ from immune guinea-pig serum was 280–320mg/100ml, which represents a threefold increase over the yield from normal serum. The yield of immunoglobulin $\gamma_1\text{G}$ from immune sera was 45–85mg/100ml and it proved impossible to isolate this protein in significant amount from normal serum. The immunoglobulin $\gamma_2\text{G}$ preparations gave a single line on immunoelectrophoresis against a polyvalent antiserum to guinea-pig serum, and the immunoglobulin $\gamma_1\text{G}$ fractions usually showed trace amounts of immunoglobulin $\gamma_2\text{G}$ or β -globulin (Fig. 4). The anti-(bovine γ -globulin) content of these preparations

Fig. 3. Immunoelectrophoresis with rabbit anti-guinea-pig serum. 1, Guinea-pig serum; 2, high-density material from euglobulin precipitate; 3, immunoglobulin γM isolated from high density material of euglobulin precipitate on Sephadex G-200 (see the text).

Fig. 4. Immunoelectrophoresis with rabbit anti-guinea-pig serum. The fractions analysed are shown in Fig. 2. 1 Guinea-pig serum; 2, pre-gradient peak (immunoglobulin $\gamma_2\text{G}$); 3, main gradient peak immunoglobulin $\gamma_1\text{G}$ (1); 4, late gradient pool immunoglobulin $\gamma_1\text{G}$ (2); 5, 32%-saturated $(\text{NH}_4)_2\text{SO}_4$ precipitate.

isolated from immune serum was 11–25% for immunoglobulin γ_2 G and 45–70% for immunoglobulin γ_1 G.

Reductive cleavage of immunoglobulins

Reduction of immunoglobulin γ M in 0.05M-cysteine (see the Materials and Methods section) transformed about 70% of the material to the monomer subunit, which was separated from unreduced or aggregated immunoglobulin γ M by gel filtration on Sephadex G-200. On immunoelectrophoresis against rabbit antiserum to guinea-pig serum, the γ M subunits showed a single 7S-type precipitin arc with mobility corresponding to that of the parent immunoglobulin γ M. Like the latter, the subunit showed a tendency to aggregate on storage at 2°C.

Polypeptide chains were prepared from immunoglobulin partially reduced with 0.3M-mercaptoethanol in 0.5M-tris-HCl buffer, pH 8.0, or completely reduced in 7M-guanidinium chloride (see the Materials and Methods section). Partially reduced alkylated immunoglobulin γ_2 G was not completely dissociated in either 0.1M-formic acid or 1.0M-acetic acid, the yield of light chain calculated from specific extinction coefficients being 20% and 25% respectively, compared with 31% when dissociated in 5M-guanidinium chloride (Table 1). After dissociation of partially reduced immunoglobulin γ_1 G in 1.0M-acetic acid, the yield of light chain was

27% (cf. 31% after complete reduction) and much of the heavy chain was aggregated (Table 1). Equilibration of partially reduced immunoglobulin γ M or γ M subunits in 1M-acetic acid resulted in precipitation of the proteins; dissociation and gel filtration were therefore carried out in 5M-guanidinium chloride. The light-chain recovery from completely reduced immunoglobulin γ M was 30%, and from γ M subunits 33%.

Properties of immunoglobulins and their constituent chains

Molecular weights. Both immunoglobulin γ M and γ M subunits showed about 10% of aggregate on ultracentrifugation. The $s_{20,w}^0$ values for immunoglobulin γ M and its subunit were about 12% higher than values reported for human monoclonal macroglobulin (Miller & Metzger, 1965) so that the estimated molecular weights of 970 000 and 191 000 were correspondingly greater (Table 2). However, the immunoglobulin γ M/subunit weight ratio (5.08:1) demonstrates the pentameric structure of guinea-pig immunoglobulin γ M. This is also shown by the correspondence between the molecular weight of immunoglobulin γ M estimated from ultracentrifugation and calculated from gel filtration of the constituent μ and light chains, assuming 10 chains of each type. The immunoglobulin γ_1 G was homogeneous on ultracentrifugation but immuno-

Table 1. *Recovery of polypeptide chains from partially and totally reduced immunoglobulins*

The immunoglobulin was 1, reduced in 0.5M-tris-HCl buffer, pH 8.0, with 0.3M- β -mercaptoethanol at about 20°C for 1 h and blocked with 10% molar excess of iodoacetamide; and 2, reduced in 0.5M-tris-HCl buffer (pH 8.2)–7M-guanidinium chloride at 37°C for 1 h and blocked with 10% molar excess of iodoacetamide in 1.0M-tris-HCl buffer–3.3M guanidinium chloride. Total recoveries are expressed in terms of total protein applied to the column and are calculated from specific extinction coefficients corrected for light scattering.

Immunoglobulin	Reduction	Dissociation and separation	Recovery from gel filtration (%)			
			Aggregate	H-chain monomer	L-chain	Total
γ M	1	5M-Guanidinium chloride; Sephadex G-200	15	59	25	86
	2	5M-Guanidinium chloride; Sephadex G-200	4	70	30	88
γ M subunit	1	5M-Guanidinium chloride; Sephadex G-200	6	61	33	80
γ_1 G	1	1.0M-Acetic acid; Sephadex G-100	73	—	27	97
	2	5M-Guanidinium chloride; Sephadex G-200	—	69	31	91
γ_2 G	1	0.1M-Formic acid; Sephadex G-100	16	64	20	97
	1	1.0M-Acetic acid; Sephadex G-100	17	58	25	99
	2	5M-Guanidinium chloride; Sephadex G-200	—	69	31	92

Table 2. *Molecular weights of guinea-pig immunoglobulins and their constituent chains*

Gel-filtration mol.wt. is derived from a plot of $\sqrt{K_D}$ against $\sqrt{\text{mol.wt.}}$ (where $K_D = \frac{V_E - V_0}{V_1}$ and V_E = peak elution volume, V_0 = excluded volume, V_1 = included volume, $V_0 + V_1$ = elution volume for small molecules). Calculation of s.d. includes s.d. in determination of the best straight line plot and s.d. for triplicate K_D determinations.

Immunoglobulin	Ultracentrifugation		Gel-filtration mol.wt. ($\pm$ s.d.)*
	$s_{20,w}^0$ (S)	Mol.wt.	
γ M	19.43	970 000	930 000* $\pm$ 60 000
γ M subunit	7.26	191 000	186 000* $\pm$ 12 000
γ_2 G	6.84	161 000	151 000* $\pm$ 10 000
γ_1 G	6.99	165 000	150 000* $\pm$ 10 000
μ chain			70 000 $\pm$ 4 000
γ_2 chain			53 000 $\pm$ 3 000
γ_1 chain			52 000 $\pm$ 3 000
Light chain			22 000 $\pm$ 2 000

* Calculated from mol. wt. of heavy and light chains.

Table 3. *Extinction coefficients of immunoglobulins and their chains*

Immunoglobulin	$E_{280}^{1\%,1\text{cm}}$	
	Measured	Corrected for light-scattering
γ M	11.8	10.5
γ_2 G	13.2	12.3
γ_1 G	13.8	13.0
γ_2 chain	13.4	13.2
γ_1 chain	14.4	13.5
μ chain	11.6	9.9
Light chain	12.5	12.0

Table 4. *Carbohydrate composition of guinea-pig immunoglobulins*

Amounts of protein analysed were determined from the specific extinction coefficients corrected for light-scattering. Results are $\pm$ s.d.

Immunoglobulin ...	Carbohydrate (g/100 g of protein)		
	γ M	γ_1 G	γ_2 G
Hexose	5.20 $\pm$ 0.035	1.19 $\pm$ 0.07	1.25 $\pm$ 0.04
Fucose	0.77 $\pm$ 0.05	0.13 $\pm$ 0.00	0.15 $\pm$ 0.01
Hexosamine	2.91 $\pm$ 0.12	1.10 $\pm$ 0.07	1.23 $\pm$ 0.10
Sialic acid	1.61 $\pm$ 0.12	0.37 $\pm$ 0.04	0.36 $\pm$ 0.01
Total	10.51 $\pm$ 0.64	2.79 $\pm$ 0.18	2.89 $\pm$ 0.16

globulin γ_2 G contained about 5% of an 11S component. This 11S material is thought to be aggregated immunoglobulin γ_2 G in equilibrium with the monomer since it could not be removed by gel filtration. Molecular weights calculated from sedimentation coefficients showed rather poor agreement with values from gel filtration of the respective heavy and light chains (Table 2).

Extinction coefficients. The measured E_{280} values

were corrected for light-scattering at 280nm by linear extrapolation of E_{315} and E_{330} values (Melamed & Green, 1963); the corrected E values were employed in amino acid and carbohydrate analyses (Table 3).

Carbohydrate composition. This showed close correspondence for immunoglobulins γ_1 G and γ_2 G, but considerably higher values were obtained for immunoglobulin γ M (Table 4).

Table 5. *Amino acid composition (residues/mol) of guinea-pig immunoglobulins and their chains**S*-Carboxymethylcysteine content was determined (a) after total reduction in 5*M*-guanidinium chloride; (b) after mild reduction in aqueous buffer.

Residue	Amino acid composition (residues/mol)										
	L chain	γ_1 chain	Immu- globulin γ_1 G	2L chains+ $2\gamma_1$ chains*	γ_2 chain	Immu- globulin γ_2 G	2L chains+ $2\gamma_2$ chains*	μ chain	Immu- globulin γ M	2L chains+ 2μ chains*	γ M subunit
Lys	9.9	24.3	70.3	68.4	29.3	80.4	78.4	33.0	92.1	85.8	87.9
His	3.3	7.5	20.6	21.6	8.1	22.7	22.8	12.6	34.1	31.6	32.4
Arg	7.3	12.9	39.2	40.4	15.8	45.0	46.2	18.2	52.5	51.0	52.1
Asx	16.8	38.5	111	111	35.0	104	104	47.5	130	129	131
Thr†	18.4	40.6	118	118	40.0	116	117	47.7	133	132	135
Ser†	29.3	49.0	157	157	48.2	153	155	51.7	157	162	164
Glx	22.4	36.9	118	119	37.1	122	119	51.3	142	147	150
Pro	11.9	33.4	89.0	90.6	37.3	104	98.4	31.5	85.4	86.6	89.3
Gly	15.1	32.2	92.7	94.6	28.1	86.0	86.4	40.8	118	112	114
Ala	13.3	23.3	72.1	73.2	24.8	77.4	76.4	28.4	81.7	83.4	84.9
Val‡	13.2	37.9	102	102	44.0	113	114	45.1	118	117	120
Met	1.2	8.7	19.8	19.8	7.7	17.4	17.8	5.8	16.8	14.0	14.2
Ile	9.0	17.1	53.4	52.2	16.2	50.8	50.4	28.3	73.8	74.6	76.3
Leu†	15.0	35.2	102	100	30.5	90.0	91.0	45.4	119	121	123
Tyr	10.1	15.3	51.2	50.8	16.6	53.2	53.4	16.5	47.8	53.2	53.9
Phe	6.3	17.0	48.1	46.6	16.2	44.9	45.0	21.5	55.6	55.6	56.9
Trp	3.4	12.3	31.2	31.4	12.0	30.1	30.8	10.9	29.8	28.6	—
Cys (a)	4.9	13.3	36.7	36.5	12.6	34.5	35.0	14.3	39.7	38.4	—
(b)	1.1	5.3	—	—	4.3	—	—	5.7	—	—	—
Total	212	455	1332	1334	460	1344	1343	551	1526	1527	1460

* Calculated from compositions of individual chains.

† Threonine and serine values were extrapolated to 0 h hydrolysis.

‡ Valine and leucine were determined after 72 h hydrolysis only.

Table 6. *Properties of guinea-pig immunoglobulins*

References: *a*, Binaghi (1966); *b*, Ovary, Benacerraf & Bloch (1963); *c*, Strejan & Campbell (1967, 1968); *d*, Berken & Benacerraf (1966); *e*, del Guercio, Tolone, Andrade, Biozzi & Binaghi (1969); *f*, Osler, Oliveira, Shint & Sandberg (1969); *g*, Bloch, Kourilsky, Ovary & Benacerraf (1963); *h*, Linscott & Hansen (1969); *i*, Bloch, Ovary, Kourilsky & Benacerraf (1963); *j*, Benacerraf *et al.* (1963); *k*, Oettgen, Binaghi & Benacerraf (1965); *l*, Nussenzweig, Lamm & Benacerraf (1966); *m*, Nussenzweig & Benacerraf (1966).

	Immunoglobulin			References
	γ_1 G	γ_2 G	γ M	
Structural formula	$(\gamma_1 L)_2$	$(\gamma_2 L)_2$	$[(\mu L)_2]_5$	
$s_{20,w}^0$ (S)	6.99 (6.6)	6.85 (6.3)	19.43	<i>j</i> *
Mol.wt.	165 000	161 000	970 000	
$E_{280}^{1cm,1\%}$	15.2 (15.01)	14.7 (13.23)	12.0	<i>k</i> *
Carbohydrate (g/100g)	2.8	2.9	10.5	
L-chains				
κ/λ ratio normal	2:1	2:1		<i>l</i>
anti-dinitrophenol	99:1	99:1		<i>m</i>
Mol.wt.	22 000	22 000	22 000	
$E_{280}^{1cm,1\%}$	12.8	12.8		
S-S bridges†: H-L	1	1		
Intrachain	2	2		
H-chains				
Mol.wt.	52 000	53 000	70 000	
$E_{280}^{1cm,1\%}$	15.9	15.6	11.4	
S-S bridges†: H-H (H-L)	4, (1)	3, (1)	5 (1)	
Intrachain	4	4	4	
Biological				
Serum concn. (mg/ml): Normal	0.3-0.7	7-10		<i>a</i>
Immune	8-18	10-14		<i>a</i>
Tissue sensitization	+	+	-	<i>b, c</i>
Cytophilic activity	-	+	+	<i>d, e</i>
C' fixation	+, -	+	+, -	<i>f, g, h</i>
Placental transfer	+	+		<i>i</i>

* Refers to values in parentheses.

† Estimated from *s*-carboxymethylcysteine content of fully and partially reduced proteins (see the text).

Amino acid composition. Comparison of the compositions reveals that immunoglobulin γ_2 G is richer in lysine, arginine and proline, and immunoglobulin γ_1 G in methionine and leucine (Table 5). Immunoglobulin γ M is similar to its subunit but differences in glutamic acid/glutamine, methionine and tyrosine suggest some contamination of the macroglobulin preparation. The compositions of γ_1 and γ_2 chains reflect the differences of the parent molecules and, in addition, the γ_2 chain is richer in valine and the γ_1 chain in glycine. The μ chain is distinguished from γ chains by higher concentrations (expressed as residues %) of histidine, glycine and isoleucine and lower concentrations of serine, proline, methionine, tyrosine and tryptophan. Analyses of light chains from immunoglobulins γ_1 G and γ_2 G showed almost complete identity and the measured compositions of the three whole immunoglobulins are close in each case to that calculated from two heavy and two light chains (Table 5).

DISCUSSION

The biological properties and structural features of guinea-pig immunoglobulins γ_1 G and γ_2 G are summarized in Table 6. The present study has confirmed the similarity of these two proteins with regard to molecular weights and carbohydrate content and the differences in amino acid composition (Lamm, 1969), which are compatible with their distinct electrophoretic mobilities. Purified immunoglobulins γ_1 G and γ_2 G antibody preparations with anti-dinitrophenol specificity (Lamm, 1969) were lower in methionine and higher in alanine content than the immunoglobulin γ G preparations analysed in this study and there were additional differences in serine and tryptophan (immunoglobulin γ_1 G), and histidine (γ_2 G). The differences between molecular weights determined by gel filtration and calculated from sedimentation data probably arose from the use of inappropriate values for partial specific volume and diffusion coefficient,

which were based on data for human immunoglobulins (Miller & Metzger, 1965)

The γ_1 and γ_2 chains have similar molecular weights and reflect all the chemical differences of their parent molecules. Comparison of heavy chains from non-specific immunoglobulin with γ_1 and γ_2 chains from anti-dinitrophenol preparations (Lamm, 1969) shows differences in methionine, isoleucine and tyrosine (γ_1) and histidine (γ_2). The γ_1 and γ_2 chains have similar numbers of half-cystine residues resistant to mild reduction (γ_1 , 8.0; γ_2 , 8.3) suggesting that both chains have four intrachain disulphide bonds and so conform to the pattern established for human γ -chains (Edelman *et al.* 1969). The number of half-cystine residues susceptible to mild reduction differs in γ_1 (5.3) and γ_2 (4.3) chains. Assuming that these residues form interchain disulphide bonds and that one half-cystine links with light chain, the probable number of inter-heavy-chain disulphide bridges is four in immunoglobulin γ_1 G and three in immunoglobulin γ_2 G. At least one of the labile bonds might form an intrachain disulphide bridge in the *N*-terminal region of H chains similar to that recorded in rabbit γ -chains (Palmer & Nisonoff, 1964; O'Donnell, Frangione & Porter, 1970) and in a human monoclonal γ -chain (Piggot & Press, 1967; Press & Hogg, 1969). This appears unlikely, however, since reduction of Fab fragment from guinea-pig immunoglobulin γ_1 G or immunoglobulin γ_2 G yields only one labile bond which must link heavy and light chains.

The molecular weights and amino acid composition of light chains from immunoglobulins γ_1 G and γ_2 G are closely similar. Identity of amino acid composition has been reported in light chains derived from specific anti-dinitrophenol antibodies (Lamm, 1969). Differences between non-specific and anti-dinitrophenol light chains greater than 1 residue/mol occur in histidine, serine, glycine, alanine and methionine contents, and these are probably attributable to the fact that guinea-pig anti-dinitrophenol antibodies contain κ -chains almost exclusively (Nussenzweig & Benacerraf, 1966). The light chains contain 3.8 half-cystine residues resistant to mild reduction, which suggests the presence of two intrachain disulphide bonds, an established pattern for light chains of other species (Milstein, 1966).

The physicochemical properties of guinea-pig immunoglobulin γ M have not previously been reported. The protein is a euglobulin recoverable in relatively low yield (10–35 mg/100 ml) from immune serum. The molecular weight calculated from sedimentation data is 970 000 and in common with other species, the protein has a carbohydrate content (10.5%) almost fourfold greater than that of immunoglobulin γ G. On mild reduction with

cysteine in neutral buffer the molecule dissociates into units of mol.wt. 191 000, so that guinea-pig immunoglobulin γ M is a pentamer, as in other species. The molecular weight of μ chains (70 000) is identical with that of the corresponding rabbit chains estimated by ultracentrifugation in 5M-guanidinium chloride (Lamm & Small, 1966), and the light chains have the same molecular weights as those of immunoglobulins γ_1 G and γ_2 G. Minor differences in amino acid composition of immunoglobulin γ M and γ M subunit (glutamic acid/glutamine, methionine and tyrosine), and relative yields of heavy and light chains after reduction, suggest the presence of some contaminating protein in the whole immunoglobulin γ M preparation. Differences of composition (residues %) between immunoglobulins γ M and γ_1 G or γ_2 G are reflected in the analyses of their respective heavy chains and include histidine, glycine and isoleucine (higher in μ chain) and serine, proline, methionine, tyrosine and tryptophan (lower in μ chain). The number of half-cystine residues resistant to mild reduction (8.6) suggests that μ -chains contain four intrachain disulphide bonds and in this respect resemble γ_1 and γ_2 chains. The guinea-pig μ chain has 5.7 half-cystine residues susceptible to mild reduction; assuming that these are all involved in interchain bonding, and that one is concerned with H–L and another with inter-subunit linkage (Beale & Feinstein, 1970), there appear to be, on average, four inter-heavy-chain disulphide bonds in immunoglobulin γ M.

We thank Dr G. T. Stevenson, Department of Biochemistry, University of Oxford for help with analytical centrifugation, Dr M. D. Melamed and Miss P. E. Lawrence for assistance with amino acid analyses, and Mr C. van Eyken for technical assistance. This work was supported by grants from the Nuffield Foundation and the Medical Research Council.

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