Goat Immunoglobulin G

PEPTIDE CHAINS AND TERMINAL RESIDUES

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(Received 10 June 1969)

Goat immunoglobulin G (IgG) was isolated and characterized. The molecular weights of the IgG and its heavy chains and light chains were found to be 144 000, 53 600 and 23 000 respectively. The light chain corresponds to human L type as was shown by the absence of *C*-terminal *S*-carboxymethylcysteine and its high content of *N*-terminal pyrrolid-2-one-5-carboxylic acid (PCA). The major *C*-terminal residue of the light chain was serine and the major *N*-terminal dipeptide was PCA-Ala (0.6 mole/mole). The major *C*-terminal residue of the heavy chain was glycine and the *N*-terminal sequence of the heavy chain is PCA-Val-Gln. This tripeptide was obtained in a 70% yield.

The four-chain structure of antibody molecule (Porter, 1962), consisting of two heavy chains and two light chains, has been established for all vertebrate immunoglobulins analysed so far (Cohen & Milstein, 1967). It has been suggested that the two light-chain classes that correspond to the human K and L ones are present in most mammals (Hood, Gray, Sanders & Dreyer, 1967). However, the ratio of K to L chains in mammals varies markedly from one species to another, the astiodactyls being richer in the L-type light chain (Hood *et al.* 1967). This conclusion was arrived at mainly by analysing *N*- and *C*-terminal peptides of light chain, assuming *N*-terminal PCA* and the absence of any *C*-terminal *S*-carboxymethylcysteine as typical features of the L-type light chains. Much less is known about the Fd portion of the heavy chain, but the finding of four different PCA peptides at the *N*-terminus of rabbit heavy chain was of considerable significance (Wilkinson, Press & Porter, 1966). However, these variations were found to be somehow related to allotypic specificity (Wilkinson, 1969).

In the present paper we report the isolation and characterization of goat IgG and its polypeptide chains. The four-chain model was found to be applicable also for this immunoglobulin. From analysis of *N*-terminal peptides and *C*-terminal residues it appears that goat IgG light chain is predominantly, if not completely, an L-type light

chain. Both peptide chains have *N*-terminal PCA and the first three residues in the heavy chain show a single unique sequence.

MATERIALS AND METHODS

Goat IgG and antiserum. Goat IgG was prepared from goat serum by precipitation at 33% saturation of $(\text{NH}_4)_2\text{SO}_4$ followed by chromatography on DEAE-cellulose equilibrated with 17.5 mM-sodium phosphate buffer, pH 6.3 (Levy & Sober, 1960). Mild reduction of IgG was performed as described by Fleischman, Pain & Porter (1962) except that 0.01 M-dithiothreitol was used for reduction, and iodoacetic acid was used for alkylation. Rabbits were immunized against goat serum by injecting 0.2 ml. of goat serum in complete Freund's adjuvant and against IgG by injecting 2 mg. of the IgG in complete Freund's adjuvant. Immuno-electrophoresis was performed as described by Scheidegger (1955).

Specific extinction coefficients. Specific extinction coefficients at 280 nm. were determined for goat IgG, and its heavy chains and light chains, by comparing the extinction with the weight of a known volume of their solution that had been dried *in vacuo* over P_2O_5 , similarly to that described by Crumpton & Wilkinson (1963). With the IgG this was also corroborated by Kjeldahl nitrogen analysis (Steyermark, 1961), assuming a nitrogen content of 16.5%.

Sedimentation and diffusion measurements. Measurements of sedimentation velocity were made in a Spinco model E ultracentrifuge at 59 780 rev./min. Diffusion measurements were made in the same centrifuge with a synthetic-boundary cell as described by Ehrenberg (1957). The sedimentation and diffusion measurements were carried out in 0.15 M-NaCl-10 mM-sodium phosphate buffer, pH 7.0, for IgG and in 6 M-guanidinium chloride unbuffered, pH 4.5, for heavy chains and light chains. All samples were equilibrated by dialysis against the respective solution. Measurements were performed at protein concentrations

* Abbreviations: PCA, pyrrolid-2-one-5-carboxylic acid; IgG, immunoglobulin G (nomenclature of chains and fragments is that recommended by the World Health Organisation, 1964); DNS, 1-dimethylnaphthalene-5-sulphonyl; [Glu, blocked glutamic acid.

ranging from 0.2% to 1.0%. Measured values were extrapolated to zero concentration. Appropriate corrections were made to obtain $S_{20,w}^0$ and $D_{20,w}^0$.

Amino acid analyses. Protein samples were dried over P_2O_5 and a known weight was hydrolysed in constant-boiling HCl (redistilled) *in vacuo* at 110° for 24 hr. Hydrolysates were dried at 40° in a rotary evaporator and were analysed on a Beckman model 120B amino acid analyser. Cystine content was determined also after complete reduction and carboxymethylation in 8M-urea. Tryptophan was determined by the method of Spies & Chambers (1949), procedure K, except that the analysis was scaled down tenfold.

Carbohydrate analysis. The carbohydrate content was determined by the method of Dubois, Gilles, Hamilton, Rebers & Smith (1956), with glucose as standard. The extinction was read at 490 nm. Hexosamine was determined by Blix's (1948) modification of the Elson-Morgan method, with *N*-acetylglucosamine as standard. The extinction was read at 530 nm.

Determination of terminal residues. Dinitrophenylation with 1-fluoro-2,4-dinitrobenzene was performed by the method of Porter (1957). Dansylation was done by the method of Gray & Hartley (1963). Hydrazinolysis was carried out as described by Bradbury (1956).

Enzymes and chemicals. Carboxypeptidase A was purchased from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.) and pronase from Calbiochem (Los Angeles, Calif., U.S.A.). Guanidinium chloride from BDH (Chemicals) Ltd. (Poole, Dorset) was filtered through a Millipore filter, 0.22 μ m. pore size, to remove absorbent material.

RESULTS

Isolation of IgG and separation of its polypeptide chains. The isolation of goat IgG by ammonium sulphate precipitation and DEAE-cellulose chromatography yielded 12g. of IgG/l. of serum. Fig. 1 shows the immunoelectrophoretic pattern of the isolated IgG against rabbit anti-goat serum as well as the monospecificity of serum obtained from rabbits injected with the isolated IgG. The separation of the heavy chains and light chains after mild reduction and carboxymethylation was first attempted on a Sephadex G-75 column equilibrated with 1M-propionic acid. However, the separation was not satisfactory and the chains were contaminated by each other. Hence the separation of the polypeptide chains was performed on a

Sephadex G-100 column equilibrated with 8M-urea-1M-propionic acid at room temperature (Fig. 2). The yields, relative to the extinction at 280 nm., of the heavy chains and the light chains were 69.5% and 30.5% respectively. The fractions obtained from the column were desalted on a Sephadex G-25 column equilibrated with 0.1M-acetic acid and freeze-dried.

Specific extinction coefficients and molecular weights. The specific extinction coefficients at 280 nm. were: for goat IgG 13.0, for heavy chain 12.0 and for light chain 12.7. For IgG the extinction was measured at pH 7.0, and for the heavy chain and the light chain at pH 3.0 in 0.1M-acetic acid.

The molecular weights were calculated from sedimentation velocity and diffusion data (Table 1). No aggregation was noted in the sedimentation

Fig. 2. Separation of heavy chains and light chains of goat IgG. Mildly reduced and carboxymethylated goat IgG (700 mg.) was eluted from a Sephadex G-100 column (4 cm. $\times$ 120 cm.) with 8M-urea-1M-propionic acid. Fractions of volume 14 ml. were collected.

Fig. 1. Immunoelectrophoresis pattern of goat serum (A) and goat IgG (B). Upper trough: rabbit anti-goat serum; lower trough: rabbit anti-(goat IgG).

Table 1. *Molecular weights of goat IgG and its polypeptide chains*

	$10^{13} \times S_{20}$	$10^{13} \times S_{20,w}^0$	$10^7 \times D_{20}$	$10^7 \times D_{20,w}^0$	Mol.wt.
Goat IgG*	6.8	7.0	4.5	4.6	144000
Heavy chain†	1.15	2.52	2.9	4.1	53600
Light chain†	0.84	1.84	4.9	6.9	22300

* Measurements were done in 0.15M-NaCl-10mm-phosphate buffer, pH 7.0. Partial specific volume, $\bar{v}$, was taken as 0.745.

† Measurements were done in 6M-guanidinium chloride. Partial specific volume, $\bar{v}$, was taken as 0.72 and the density of 6M-guanidinium chloride was taken as 1.14.

Table 2. *Amino acid and carbohydrate composition of goat IgG and its polypeptide chains*

The results are given as moles of amino acids/mole of protein as calculated from the recovery of amino acids from 24 hr. hydrolysates of known weights of IgG, heavy chains and light chains and assuming the molecular weights given in Table 1. SCM-Cys, *S*-carboxymethylcysteine.

	Amino acid and carbohydrate content (moles/mole)			
	Heavy chain	Light chain	2 heavy + 2 light chains	IgG
Lys	18.4	10.0	56.8	54.7
His	6.9	1.1	16.1	16.0
Arg	14.3	3.6	35.8	38.5
Asp	29.6	14.9	89.0	88.4
Thr*	37.9	19.8	115.4	118.7
Ser*	50.7	32.7	166.8	166.3
Glu	43.4	18.0	122.8	121.1
Pro	34.5	10.2	89.0	85.1
Gly	29.1	17.2	92.6	93.4
Ala	22.7	13.5	72.4	70.1
Val	40.9	15.4	112.2	115.3
Met	2.7	0.1	5.6	5.7
Glu	10.5	6.0	33.0	31.0
Leu	30.7	12.2	85.5	83.3
Tyr	16.7	8.6	50.6	48.4
Phe	13.8	4.2	36.0	35.4
Cys	6.5	3.3	19.6	32.6†
SCM-Cys	5.5	1.0	13.0	—
Trp	6.5	1.9	16.8	19.9
Hexose	6.7	0.5	14.4	13.3
Hexosamine	4.6	0.5	10.2	11.5

* Not corrected for decomposition during hydrolysis.

† This value was obtained from the sum of the *S*-carboxymethylcysteine contents of fully reduced and alkylated heavy chains and light chains.

pattern of either heavy chains or light chains in 6M-guanidinium chloride. Results obtained were close to those reported for immunoglobulins of other species (Cohen & Milstein, 1967).

Amino acid and carbohydrate analysis. Table 2

gives the amino acid and carbohydrate content of goat IgG and its polypeptide chains. The significant features are the low content of methionine in IgG and the apparent absence of methionine from the light chain. Other differences between the amino acid contents of the chains are the relatively low content of histidine, arginine, proline and *S*-carboxymethylcysteine in the light chain. It seems that there are two intrachain disulphide bonds in the light chains, as in human IgG (Milstein, 1966), and three or four intrachain disulphide bonds in the heavy chain in addition to those reduced under the conditions used for separation of the chains.

N-Terminal residues of polypeptide chains. Dinitrophenylation of 0.2 μ mole of heavy chains or of light chains did not show any α -DNP-amino acids in either the ether phase or the water phase. Hence a search was made for a blocked *N*-terminal residue by the method used by Press, Piggot & Porter (1966). The heavy chain was fully reduced and carboxymethylated in 8M-urea and dialysed exhaustively against water. The fully reduced and alkylated heavy chain was completely soluble in water. Then 1 μ mole was digested with pronase at pH 8.2 at 37° (enzyme/protein ratio 1:25, w/w) in the cell of the pH-stat. Digestion was complete after 3 hr. The digest was applied to a 1 cm. $\times$ 10 cm. column of Dowex 50 (X2; H⁺ form; 200–400 mesh) and eluted with water at room temperature. The elution of peptides was followed by measuring the extinction at 215 nm. Only one peak was obtained. Amino acid analysis of 5% of the eluate after acid hydrolysis showed glutamic acid and valine in the ratio 1.9:1, and the yield of valine was 0.7 mole/mole of heavy chain; other amino acids were also found but their content was less than 10% of that of valine. High-voltage electrophoresis of the eluate at pH 3.5 revealed only one peptide, which was detected by the hypochlorite-starch-iodide technique (Pan & Dutcher, 1956), with a mobility of 0.44 compared with that of PCA. Analysis of this peptide showed again the composition (Glu_{2.1}, Val_{1.0}). Hydrazinolysis of the peptide did not yield any free amino acid. Carboxypeptidase A digestion (0.1 μ mole of peptide to 15 μ g. of

carboxypeptidase A in 0.1M-ammonium hydrogen carbonate for 24 hr. at 37°) yielded only glutamine (95% yield). This was detected both as a peak in the position of serine on the long column of the amino acid analyser and as DNS-glutamine after dansylation without hydrolysis. Hydrazinolysis after carboxypeptidase digestion showed only valine (50% yield). Hence the sequence of this peptide is [Glu-Val-Gln. No attempt was made to determine the nature of the blocking group of the *N*-terminal glutamic acid, but by analogy with the investigation of the blocked *N*-terminal residue of rabbit heavy chains and light chains (Wilkinson *et al.* 1966; Rüde & Givol, 1968) and of human heavy chain (Press *et al.* 1966) we assume that the blocked residue is PCA and that the sequence of the *N*-terminal peptide is PCA-Val-Gln. The light chain was treated similarly except that it was not fully reduced and alkylated before pronase digestion. Analysis of 5% of the eluate from the Dowex 50 column showed that the predominant amino acids were glutamic acid and alanine in the ratio 1.3:1. The recovery of alanine was 0.6 mole/mole of light chain. After paper electrophoresis at pH 3.5 one major peptide was obtained (mobility 0.54 compared with that of PCA) and its composition was (Glu_{1.1}, Ala_{1.0}). Hydrazinolysis showed the *C*-terminal residue to be alanine (55% yield). Hence the sequence must be [Glu-Ala and is probably PCA-Ala.

***C*-Terminal residues.** The *C*-terminal residues of the heavy chains and light chains were determined by hydrazinolysis. The major *C*-terminal residue in the heavy chain is glycine (0.67 mole/mole), similar to rabbit IgG (Givol & Porter, 1965), but serine (0.25 mole/mole) is also present. In the light chain the major *C*-terminal residue is serine (0.5 mole/mole), similar to human L-type chain (Milstein, 1965), but glycine (0.35 mole/mole) and alanine (0.12 mole/mole) are present. No traces of *S*-carboxymethylcysteine (the *C*-terminal residue of human K-type light chain) were found.

DISCUSSION

Under the conditions employed, an average of 6.5 disulphide bonds were reduced/mol. in the goat IgG, similarly to the situation in rabbit IgG (Fleischman, Porter & Press, 1963). The *S*-carboxymethylcysteine contents were 1.0 and 5.5 moles/mole in light chain and heavy chain respectively. The separation of reduced and alkylated peptide chains on Sephadex G-100 in 8M-urea-1M-propionic acid resulted in the recovery of heavy chains and light chains with mass yields of 70.6% and 29.4% respectively, which are exactly in a 1:1 molar ratio (Fig. 2). This, together with the finding of one *N*-terminal residue (PCA) in significant yields for both chains, and the molecular weights of the chains and the parent IgG molecule (Table 1), provides evidence for a four-chain molecule composed of two heavy chains and two light chains in goat IgG.

The heterogeneity of *C*-terminal residues in the heavy chain and light chain, both containing mainly serine and glycine, could not be due to cross-contamination of the isolated chains, since no such cross-contamination was detected in the *N*-terminal peptides. It is possible that this heterogeneity is due to the presence of subclasses in the IgG that is reflected in the constant *C*-terminal part. Such heterogeneity of *C*-terminal residues was found also in rabbit light chain (Givol & De Lorenzo, 1968), but not in rabbit heavy chain (Givol & Porter, 1965). The absence of *C*-terminal *S*-carboxymethylcysteine could not be attributed to the failure of the method employed, since this residue was obtained in good yield by hydrazinolysis of rabbit IgG light chain. This indicates that goat IgG light chain is homologous to the human L-type light chain, which has *C*-terminal serine (Milstein, 1966; Putnam, 1969). This conclusion is also supported by the finding of an *N*-terminal peptide PCA-Ala in 60% yield and probably other PCA-peptides in smaller yields that were not isolated.

Table 3. Comparison of *N*-terminal three residues in heavy chains from different sources

Heavy chain	<i>N</i> -Terminal sequence	Yield	Reference
Human Ou μ	PCA-Val-Thr		Wikler, Kohler, Shinoda & Putnam (1969)
Human Daw γ	PCA-Val-Thr		Press <i>et al.</i> (1966)
Human Eu γ	PCA-Val-Gln		Gottlieb, Cunningham, Waxdol, Konigsberg & Edelman (1968)
Human pooled γ	PCA-Val-Gln	40%	Press & Piggot (1967)
Goat pooled γ	PCA-Val-Gln	70%	This paper
Rabbit pooled γ	PCA—Ser		Wilkinson (1969)
Rabbit pooled γ	PCA-Glu-Gln		Wilkinson (1969)

The significant yield (70%) of the *N*-terminal tripeptide PCA-Val-Gln isolated from the heavy chain suggests that, in contrast with rabbit IgG heavy chain, the *N*-terminus of goat heavy chain is quite homogeneous. The comparison of the first three residues of different heavy chains from different species and classes are given in Table 3. The striking homology at the *N*-terminus of the heavy chain in different species and immunoglobulin classes is a strong indication of the evolutionary relationship of heavy chains from different species and from the IgG and macroglobulin (IgM) classes. It might also indicate that the region involved in antibody specificity does not include the first three residues from the *N*-terminus of the heavy chain.

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