Sequence Studies of the Fd Section of the Heavy Chain of Rabbit Immunoglobulin G

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A partial amino acid sequence was given by Cebra, Steiner & Porter (1968b) of the *N*-terminal half of the heavy chain of rabbit immunoglobulin G. This was extended and in part corrected to give a continuous sequence of 136 residues, which together with other work accounts for three-quarters of the total sequence. Evidence is given suggesting that there is a limited region of 10–15 residues that are exceptionally variable in the heavy chains from pooled rabbit immunoglobulin G.

The isolation has been described of the *N*-terminal half of the heavy chain of rabbit IgG* after splitting at the methionine residue near the centre of the chain by using cyanogen bromide (Givol & Porter, 1965). This fraction, named C-1, is heterogeneous owing to the presence of different sequences related to the allotypic variants (Prahl & Porter, 1968; Wilkinson, 1969), but appears to be less complex than originally expected for a section that contains the variable region of the heavy chain. It was reported that, after blocking of the lysine groups with trifluoroacetic acid, tryptic hydrolysis at the arginine residues gave six peptides that appeared to account for most of the *N*-terminal 240 residues contained in fraction C-1 (Cebra, Givol & Porter, 1968a). The partial sequence of two of the largest tryptic peptides T2 and T3 has been given (Cebra, Steiner & Porter, 1968b). We now extend and correct some of these data and give what appears to be the predominant sequence of the *C*-terminal 136 residues of the fraction C-1.

MATERIALS AND METHODS

Rabbit IgG and heavy chains were prepared as described by Prahl & Porter (1968). Enzymes and other materials used were those described by Cebra *et al.* (1968b).

The isolation of fraction C-1, its total reduction and tryptic hydrolysis after reaction with *S*-ethyl trifluoroacetate were all carried out as described by Cebra *et al.* (1968a), as was the isolation of the tryptic peptides T1–T7. Analysis of these isolated peptides agreed well with the values given by Cebra *et al.* (1968a). An alternative preparation of fraction C-1 was made as follows, in order to dissociate peptides originating from the Fc fragment that remained adsorbed on fraction C-1. After

reaction of 350 mg of heavy chain with CNBr as described by Cebra *et al.* (1968a), the mixture was dialysed against 6M-urea–0.2M-sodium formate, pH 3.2, and was fractionated on a column (140 cm × 3.0 cm) of Sephadex G-100 in the same solvent.

Enzymic digestion of peptides and paper electrophoresis were carried out as described by Cebra *et al.* (1968b).

Isolation of arginine peptides from a chymotryptic hydrolysis of fraction C-1. Fraction C-1 (150 mg) was reduced in 6M-guanidine and the thiol groups were allowed to react with iodo[2-¹⁴C]acetate (Cebra *et al.* 1968a). It was dialysed against water and the solution (about 10 ml) was brought to pH 8.3 in a pH-stat at 37°C. Chymotrypsin (2 mg) was added over 2 h and the pH maintained constant for 4 h, when the digestion appeared to be complete. The pH was raised to 9 and the temperature was lowered to 20°C, and 375 mg of maleic anhydride was added over 30 min with the pH being maintained at 9. The clear solution was poured through a column (25 cm × 1.5 cm) of Dowex 50 (X2; H⁺ form) and the column washed with water until no further elution of material absorbing at 280 nm could be detected. The adsorbed peptides, containing 90% of the arginine of the digest, were eluted with m-NH₃. This fraction was freeze-dried and dissolved in an aqueous solution of pyridine (1%, v/v) and acetic acid (10%, v/v), pH 3.5, and kept at 60°C for 16 h to remove maleyl groups (Butler, Harris, Hartley & Leberman, 1967). It was fractionated on a column (200 cm × 3.0 cm) of Sephadex G-50 in 0.05M-NH₃.

RESULTS

Completion of the sequence of peptide T2, the C-terminal tryptic peptide of fraction C-1. Cebra *et al.* (1968b), reported that after removal of trifluoroacetyl groups from the lysine residues of peptide T2 and further tryptic hydrolysis two large peptides T-T2-LB and Ts2 and two small peptides T-T2-SN and T-T2-SA could be isolated. The peptides were aligned in the order peptides T-T2-LB, T-T2-SN, Ts2 and T-T2-SA, and the sequence was completed

* Abbreviations: IgG, immunoglobulin G; CMCys (in amino acid sequences), carboxymethylcysteine.

† Gel filtration in 0.05 M-NH₃ on Sephadex G-25.

Table 2. Amino acid composition of constituent peptides of peptide T-T2-LA

	Composition (mol of amino acid residue/mol of peptide)											
	Tryptic peptides					Chymotryptic peptides					Pronase peptides	
	T-T2-LA1	T-T2-LA2	T-T2-LTA1	T-T2-LTA2	T-T2-LB	C-T2-LB-LAH	C-T2-LB-A	C-T2-LB-H	P-T2-C	P-T2-H		
Lys	1.5	1.1			1.1							
His	0.7	0.7			0.8					1.0		
CMCys	+ve	+ve			0.7				0.6			
Asp	1.7	1.9			2.2			1.1	1.1			
Thr	3.5	3.4			3.6			1.2	0.9			
Ser	6.4	6.7		2.0	5.1			2.8				
Glu	2.0	2.1	1.0	1.0	1.0			0.93				
Pro	2.7	2.6			2.7			1.4				
Gly	1.7	1.4	1.5	1.0								
Ala	2.1	2.3			2.0					1.8		
Val	3.3	3.5			4.0							
Leu	1.0	0.9	1.5	1.4				1.9				
Tyr	0.7	0.8	0.9	0.8								

tides T-T2-LA1 and T-T2-LA2 consisted of peptide T-T2-LB together with six more residues, including tyrosine. Peptide T-T2-LA1 was shown by the dansyl-Edman method to have the *N*-terminal sequence Glx-Ser; peptide T-T2-LA2 had no detectable *N*-terminal amino acid. This suggests that there was an *N*-terminal glutamine residue that cyclized in part to give pyrrolidonecarboxylic acid and hence gave two forms of the same peptide. Two further small peptides containing tyrosine were also isolated; peptides T-T2-LTA1 and T-T2-LTA2. Again their compositions were identical, but their mobilities at pH 3.5 were +0.63 and -0.02 respectively. The sequence was shown to be Gln-Ser-Ser-Gly-Leu-Tyr by using carboxypeptidase digestion as well as the dansyl-Edman method, and it appears that the whole *N*-terminal tryptic peptide occurred in four forms as a result of partial cleavage by trypsin of the Tyr-Ser bond and the partial cyclization of the *N*-terminal glutamine residue (Fig. 1).

Peptide T-T2-LB was isolated and its sequence partially determined as shown in Fig. 1. The peptides C-T2-LBA and C-T2-LBH were isolated from a chymotryptic digest of peptide T2, derived from fraction G1 that had been totally reduced and allowed to react with iodo[2-¹⁴C]acetate as described by Cebra *et al.* (1968a), together with a peptide C-T2-LBAH whose composition equalled the sum of the other two. The *N*-terminal sequences of these peptides are shown in Fig. 1, and the subsequent isolation of peptides P-T2-C and P-T2-H from pronase digestion completed the sequence of peptide C-T2-LBA and also that of peptide C-T2-LBH, except that a clear arrangement of Thr-Asx and of Pro-Ser was not obtained.

The sequence of peptide T-T2-LA with the methods of derivation is shown in Fig. 1, which summarizes the sequence and constituent peptides of this peptide and hence completes the sequence of peptide T2.

Extension of the sequence of peptide T3, the second large peptide from the C-terminal half of fraction C-1. In the partial sequence of peptide T3 reported by Cebra *et al.* (1968b), only the N- and C-terminal residues of the C-terminal tryptic peptide T-T3-LY were reported, the overlap of peptide Ts3 with peptide T-T3-LY had not been obtained and the N-terminal tryptic peptide T-T3-LB had had its sequence only partially determined and appeared to be incomplete. Most of this work has now been finished, but it appears that the N-terminal section (10-15 residues) of peptide T3 is heterogeneous and a satisfactory sequence has still not been obtained.

Tryptic digestion of peptide T3 and investigation of peptide T-T3-LY. Only peptides giving additional information are discussed. After removal of trifluoroacetyl groups from the lysine residues,

Table 3. *Amino acid composition of constituent peptides of peptide T3*

	Composition (mol of amino acid residue/mol of peptide)							
	Tryptic peptides			Chymotryptic peptides				
	T-T3-LY	T-T3-LY-V	T-6b	C-T3-LY-A	C-T3-LY-B	C-T3-LY-C	C-T3-A	C-T3-H
Lys							0.9	1.0
Arg	1.0		1.0		0.6	1.0		
CMCys							0.7	1.9
Asp	2.2	1.0	1.1		1.8	1.2		1.3
Thr	3.8	1.8	2.0	1.2	1.9	1.0		3.8
Ser	1.5		1.1		1.0			5.0
Glu	1.5	1.1		0.9				1.1
Pro	1.9	1.6		1.8				4.6
Gly	3.4	1.7	2.1		2.4	1.3	2.3	2.4
Ala								2.1
Val	3.2	1.9	1.1	1.8	1.0	1.0	1.3	3.2
Leu	2.3	1.3	1.2	1.0	1.1		1.1	2.0
Tyr	1.1	1.0					1.0	
Phe								1.0
Trp		+ve		+ve				

Table 4. *Isolation of constituent peptides of peptide T3*

Elution volumes are given relative to exclusion volume=1.0. Mobilities are given relative to arginine +1, aspartic acid -1 and ϵ -DNP-lysine 0.

		Sephadex-gel filtration in 0.05 M-NH ₃		Paper electrophoresis	
Peptide	<i>N</i> -Terminus	Type of gel	Elution volume	pH	Mobility
Tryptic peptides of peptide T3					
T-T3-LY	Gly	G-50	2.2	9.2	-0.35
T-T3-LY-V	Gly	G-50	2.5		
Chymotryptic peptides of peptide T3					
C-T3-LY-A	Leu	G-50	2.3	6.5	-0.23
		G-25	1.5		
C-T3-LY-B	Asx	G-50	2.3	6.5	Neutral
		G-25	1.5		
C-T3-LY-C	Thr	G-50	2.3	6.5	Neutral
		G-25	1.5		
C-T3-A	Gly	G-50	2.3	6.5	Neutral
		G-25	1.5		
C-T3-H	Val	G-50	1.7	3.5	-0.15

peptide T3 was digested with trypsin and fractionated on a Sephadex G-50 column, and the peptide T-T3-LY was isolated as described by Cebra *et al.* (1968b). Its analysis agreed with the earlier values and the dansyl-Edman method gave the *N*-terminal pentapeptide sequence (Fig. 2). A second smaller peptide T-T3-LY-V, also absorbing at 280nm, was isolated and analysed (Tables 3 and 4) and proved to have the same *N*-terminal sequence, and hence was judged to be the *N*-terminal section of peptide T-T3-LY.

Chymotryptic digestion of peptide T3. The purification of the chymotryptic peptides is given in Table 4

and their analysis in Table 3. Peptide C-T3-LY-A, whose sequence is shown in Fig. 2, is derived from peptide T-T3-LY. Peptide C-T3-LY-B contains arginine and hence is *C*-terminal and gave the *N*-terminal sequence Asx-Ser. Peptide C-T3-LY-C is a smaller *C*-terminal peptide and gave the sequence of the *C*-terminal pentapeptide. The overlap of the tryptic peptides Ts3 and T-T3-LY was given by the peptide C-T3-A, whose sequence was determined as shown.

The completion of the sequence of peptide

Fig. 3. Elution diagram of the arginine-enriched fraction from Dowex 50 (X2) of a chymotryptic digest of fraction C-1 (labelled with iodo[2-¹⁴C]acetate) on a column (220 cm × 3 cm) of Sephadex G-50 in 0.05 M-NH₃. — · — ·, E_{215} ; —, E_{280} ; · · ·, radioactivity (c.p.m./ml of eluate). The fractions taken were named as shown.

Table 5. Isolation procedures for chymotryptic peptides of fraction C-1

Elution volumes are given relative to exclusion volume =1.0. Mobilities are given relative to arginine +1.0 and ϵ -DNP-lysine 0.

Peptide	N-Terminus	Sephadex-gel filtration on G-50 in 0.05 M-NH ₃ Elution volume	Paper electrophoresis mobility at pH 3.5
C-C-1-5a	Thr	2.5	+0.26
C-C-1-6a	Cys	2.8	+0.18
C-C-1-6c		2.8	+0.08

T-T3-LY was achieved by the isolation of a peptide T6b from the tryptic digest of the trifluoroacetate-treated fraction C-1 during the preparation of peptide T3 and other peptides. Peptide T6b apparently arises from a chymotryptic split near the C-terminus of peptide T3, but surprisingly after an asparagine residue, not after the adjacent tryptophan residue. Its occurrence explained the presence of peptide T-T3-LY-V, the complementary half of peptide T-T3-LY, and also the low yields of the C-terminal peptides from chymotryptic digests of peptide T3. The sequence of peptide T6b is given in

Table 6. Amino acid composition of chymotryptic overlap peptides of fraction C-1

	Composition (mol of amino acid residue/mol of peptide)		
	C-C-1-5a	C-C-1-6a	C-C-1-6c
Arg	1.0	1.0	1.1
CMCys		+ve	+ve
Asp	1.1		0.7
Thr	1.4		
Ser			0.8
Glu			0.8
Gly	1.2	1.1	1.3
Ala		1.3	1.2
Val	0.9		
Tyr		0.9	1.0
Phe	0.9	0.9	0.8

Fig. 2. The satisfactory overlaps with other peptides complete this section. The large peptide C-T3-H reported by Cebra *et al.* (1968b) was reisolated and a more extensive sequence, determined by the dansyl-Edman technique, gave a slight correction: Val-Ser-Glx-Pro-Ser-Gly-Thr-Lys, not Val-Gly-Glx-Pro(Thr,Ser,Ser)Lys as reported by Cebra *et al.* (1968b). No further extension of sequence at the N-terminal end of peptide T3 could be obtained,

and as reported below other evidence suggests that there is considerable heterogeneity in this section.

Chymotryptic digest of fraction C-1. In order to align the main tryptic peptides obtained from the digestion of fraction C-1 after reaction with *S*-ethyl trifluorothioacetate, it was necessary to isolate peptides containing arginine from a chymotryptic digest. These would be expected to give the overlapping sequences that were required. As described in the Materials and Methods section, the arginine-containing peptides from the digest were concentrated by allowing the amino groups to react with maleic anhydride and running them through a Dowex 50 (H^+ form) resin column in water. About 90% of the arginine was retained on the column, but only 40% of the total amino acids (as judged by analysis of samples), and these were eluted with *m*-ammonia. After removal of maleyl groups the peptides were fractionated on a column of Sephadex G-50 in 0.05*M*-ammonia. The elution diagram is shown in Fig. 3. Peptide C-C-1-5a was isolated (Tables 5 and 6) from fraction C-C-1-5, and the *N*-terminal residue was threonine and the *C*-terminal sequence Thr-Phe. Tryptic digestion gave two peptides C-C-1-5a1 (Thr-Phe) and C-C-1-5a2 [Thr(Asp,Gly,Val)Arg]. The sequence of the whole peptide was Thr(Asp,Gly,Val)Arg-Thr-Phe. This agrees with the *C*-terminal sequence of peptide T3 and the *N*-terminal sequence of peptide T7.

A satisfactory overlapping peptide for peptides T7 and T2 has not been obtained. Several peptides derived from this section were isolated, but all terminated in arginine, and it appears that this residue is particularly labile to the traces of trypsin in the chymotrypsin preparation used. The homology of the sequence of this part is so close between human $\gamma 1$ -chain and the rabbit γ -chain (Fig. 6) that it is very unlikely that there is any other intervening peptide.

Other arginine-containing peptides were isolated and in particular the peptide containing the arginine residue adjacent to the *N*-terminal residue of peptide T3. It was hoped to gain information in this way about the *N*-terminal sequence of peptide T3. Comparison of the sequence in this area with that reported for human myeloma proteins suggests that the arginine residue would be characterized by adjacent half-cystine and aromatic residues. Such a peptide, Ts4, had been isolated from a tryptic digest of fraction C-1 (O'Donnell, Frangione & Porter, 1969), and contained the *C*-terminal sequence Tyr-Phe-Cys-Ala-Arg (Fig. 6). The equivalent chymotryptic peptide was found in fraction C-C-1-6.

Five radioactive peptides were found in this fraction and two were isolated as shown in Table 5 and their analysis is given in Table 6. Tryptic digests were done on peptides C-C-1-6a and C-C-1-6c, which showed the presence of the peptide Cys-Ala-Arg in

both. Carboxypeptidase digestion of peptide C-C-1-6a gave: Tyr (1), Phe (0.7). This would give the sequence for peptide C-C-1-6a as Cys-Ala-Arg-Gly-Phe-Tyr. The yields of the other peptides were insufficient to characterize them further other than to prove that the radioactive peptide after tryptic digestion had the mobility of Cys-Ala-Arg.

Deletion of peptide T4 from the sequence of fraction C-1. In the earlier work (Cebra *et al.* 1968a), peptide T4 was isolated in yields comparable with those of other major fractions from a tryptic digest of fraction C-1 after reaction with *S*-ethyl trifluorothioacetate. It had a high content of dicarboxylic acids and some tryptophan, similar to the section with residues 45–75 in the sequence reported for the Fd fraction of a human pathological IgG molecule (Press, 1967). For this reason it was suggested that it might have been derived from the equivalent section of the rabbit γ -chain. When, however, its sequence was investigated the similarity was less obvious, and in particular the sequence around the half-cystine residue Val-Thr-Cys-Val is identical with that reported about the Fc fraction 'N-terminal loop' of a human $\gamma 1$ myeloma protein by Frangione & Milstein (1967). Examination of the partial sequence of the Fc section of rabbit IgG (Lebovitz, Delaney, Fellows & Hill, 1968) showed that a tryptic peptide (T23) had been isolated and analysed, but its sequence not determined, whose

Fig. 4. Fractionation of a CNBr digest of rabbit heavy chain on a column (140 cm $\times$ 3.0 cm) of Sephadex G-100 in 6*M*-urea–0.2*M*-sodium formate, pH 3.2. —, E_{280} . Naming of the fractions are as shown.

Thr-Pro-Glu-Val-Thr-Cys-Val-Val-Val-Asp-Val-Ser-Glx-Asx(Asx,Pro,Glx,Val,Glx)Phe-Thr-Trp-Tyr-Ile(Asx,Asx,Glx)Gln-Val-Arg
 →→→→→→→→

Fig. 5. Partial amino acid sequence of tryptic peptide T4 showing the peptides derived from it. Sequence determination from the *N*-terminal end was by the dansyl-Edman technique, shown by →, and from the *C*-terminal end by hydrolysis with carboxypeptidase A, shown by ←.

Table 7. Isolation procedures for component peptides of peptide T4.

Mobilities are given relative to aspartic acid -1.0, ϵ -DNP-lysine 0 and arginine +1.0. Elution volumes are given relative to exclusion volume=1.0. Chromatography (descending) was carried out on paper with BAW [butan-1-ol-acetic acid-water (12:3:5, by vol.)] as solvent.

Peptide	<i>N</i> -Terminus	Sephadex gel filtration in 0.05M-NH ₃		Paper electrophoresis or chromatography	
		Type of gel	Elution volume	pH	Mobility
Chymotryptic peptides from peptide T4					
C-T4-a	Thr	G-50	1.4	6.5	−0.80
C-T4-b2	Ile	G-50	2.3	3.5	+0.50
C-T4-c1	Thr	G-50	2.9	BAW	
C-T4-b1	Tyr	G-50	2.1	3.5	+0.29
C-T4-c2	Thr	G-50	2.9	BAW	
Papain peptides from peptide C-T4-a					
Pa-C-T4-a1	Thr			6.5	−0.40
Pa-C-T4-a2	Cys			6.5	−0.75
				3.5	−0.21
Pa-C-T4-a3	Val			6.5	−0.80
				3.5	−0.10
Peptic peptides from peptide T4					
Pe-T4-1	Glu	G-25	1.7	3.5	+0.78
Pe-T4-2	Phe	G-25	1.8	BAW	

composition was similar to but not identical with that of peptide T4. This raised the possibility that this section of the Fc fraction from the cyanogen bromide digest was adsorbed strongly on the C-1 fraction, although other sections were not. An alternative preparation, which avoided freeze-

drying of the cyanogen bromide digest and used subsequent fractionation on a Sephadex G-100 column in 6M-urea-0.2M-sodium formate, was tried. The elution pattern is shown in Fig. 4. The origin of the fraction was investigated by comparison of the peptides containing half-cystine residues with those

Table 8. Analysis of component peptides of peptide T4

Composition (mol of amino acid residue/mol of peptide)											
Chymotryptic peptides of peptide T4					Papain peptides of peptide C-T4-A				Peptic overlap peptides of T4		
C-T4-a	C-T4-b1	C-T4-b2	C-T4-cl		Pa-C-T4-a1	Pa-C-T4-a2	Pa-C-T4-a3		Pe-T4-1	Pe-T4-2	
Arg	1.0	1.0							1.0		
Asp	2.2	2.0	1.0			1.0	2.0			1.0	
Thr					1.8						
Ser							1.2				
Glu	2.3	2.1			1.0		2.9		1.2		
Pro					0.86		0.9				
Val	1.1	0.94			1.1	2.65	1.9		1.1		
Ile	1.0	0.96									
Tyr	0.70			0.84							
Phe											
CMCys	1.0						0.9				0.85
Trp (Ehrlich stain)	0.67					0.32					+ve

present in the Fc fraction and the whole heavy chain. This was done by total reduction and reaction with iodo[2-¹⁴C]acetate, followed by digestion with trypsin and chymotrypsin and fractionation of the peptides by electrophoresis on paper at pH3.5. Radioautography showed the position of the radioactive peptides. With this technique it was clear that peptides C-3, C-4 and C-5 were derived from Fc fraction, fraction C-1 contained no significant content of Fc peptides and peptides CA and CB were products of partial splitting at methionine residues or of aggregates persisting in 6M-urea-0.1M-formate solution.

When this purified C-1 preparation was allowed to react with *S*-ethyl trifluorothioacetate and digested with trypsin as described by Cebra *et al.* (1968b), the elution diagram on Sephadex G-50 in 0.05M-ammonium hydrogen carbonate was similar to that obtained by Cebra *et al.* (1968b) except for an appreciable decrease in the relative size of the fraction containing peptide T4. Further resolution by chromatography on DEAE-Sephadex A-25 as described by Cebra *et al.* (1968b) gave a yield of less than 5% of peptide T4 on a molar basis.

The partial sequence of peptide T4 (Fig. 5) was derived as follows. The dansyl-Edman technique gave the *N*-terminal hexapeptide sequence Thr-Pro-Glu-Val-Thr-Cys. Chymotryptic digestion gave three main peptides, which were peptides C-T4-a, C-T4-b2 and C-T4-cl, isolated as shown in Table 7 and their analysis is given in Table 8. Their analysis accounted for the whole peptide T4. Peptide C-T4-a had *N*-terminal threonine and contained radioactive cystine, and was therefore *N*-terminal; the *C*-terminal residue was phenylalanine. Peptide C-T4-b2 had *N*-terminal isoleucine and contained arginine, and therefore was *C*-terminal, with the tripeptide C-T4-cl assumed to be between peptides C-T4-a and C-T4-b2. Peptide C-T4-cl had the sequence Thr-Trp-Tyr and its position before peptide C-T4-b2 was confirmed by the isolation of another chymotryptic peptide C-T4-b1, which had *N*-terminal Tyr-Ile instead of isoleucine but which was otherwise identical with peptide C-T4-b2. The corresponding dipeptide C-T4-c2 (Thr-Trp) was also isolated.

Peptide C-T4-a. The sequence of this large *N*-terminal peptide was extended by isolation of peptides Pa-C-T4-a1, Pa-C-T4-a2 and Pa-C-T4-a3 from a papain digest. They could be aligned from the known *N*- and *C*-terminal sequences and, in all, 15 of the 20 residues were placed.

The overlap of peptide C-T4-a with peptide C-T4-cl was given by isolation of the tripeptide Pe-T4-2, Phe(Thr,Trp), from a peptic digest of peptide T4. Another peptic peptide, Pe-T4-1 (Gln-Val-Arg), extended the *C*-terminal sequence of peptide T4.

The partial sequence obtained shows that, if maximum homology is assumed, 22 of the 30 positions are identical with the sequence reported for residues 256–285 in the heavy chain of human $\gamma 1$ pathological protein Eu (Edelman *et al.* 1969). Hence it appears certain that peptide T4 is a peptide from the Fc section of the heavy chain, which is adsorbed strongly on fraction C-1 after digestion with cyanogen bromide, and that it does not form a part of the sequence of fraction C-1 itself.

DISCUSSION

The partial sequence of peptide T3 and the complete sequences of peptides T7 and T2 account for the C-terminal 134 residues of the C-1 fraction. The homology between this sequence and that of the γ -chain of Daw pathological human IgG proteins (Press & Hogg, 1969) is shown in Fig. 6. Numbering is taken from the sequence of Daw protein. The homology is sufficiently close that the alignment of peptides T7 and T2 seems clear, although a satisfactory overlapping peptide could not be obtained.

If five deletions in the rabbit γ -chain relative to the human $\gamma 1$ -chain are assumed, to give maximum homology, then of the 135 residues of the apparent constant section 76 (or 56%) are common. There are blocks of identical residues that are particularly obvious between residues 226 and 253, where only a tripeptide deletion and three single residue changes occur, and again from residues 122 to 132 the sequence is identical except for a change from glycine to alanine at residue 124. The latter section aligns the N-terminal end of the sequence and suggests that peptides in significant yield have been obtained from residue 109 (N-terminal residue of peptide T-T3-LB) on the Daw-protein numbering. Comparison of sequences of Daw protein with Cor protein (Press & Hogg, 1969) and with Eu protein (Edelman *et al.* 1969) places the change from the variable to constant sections at residues 116 and 117 on the Daw-protein numbering, so that peptides have been obtained from a part of the chain that includes the C-terminal seven residues of the variable section by this criterion. Of the 110 residues in the N-terminal section of the γ -chain the sequence of the first 34 has been reported (Wilkinson, 1969) and has been shown to contain residues that differ between the Aa1 and Aa3 allotypes.

A peptide Ts4 judged by homology (Fig. 6) to come from the position residues 85–99 has been isolated in good yield and its sequence determined (O'Donnell *et al.* 1969). Evidence obtained in the present paper shows that one sequence in poor yield could be established from residues 100 to 104. Other peptides apparently derived from different sequences in the same section were also isolated, but in such

Rabbit Daw	85	Ile	Thr	Ser	Pro	Thr	Gln	Asp	Thr	Ala	Thr	Tyr	Phe	Cys	Ala	Arg	100	Asx	Leu	(Gly, Gly, Leu, Val, Thr)	Val	Ser	Glx	Ser														
	90	Asn	Thr	Val	Gly	Pro	Gly	Asp	Thr	Ala	Thr	Tyr	Tyr	Cys	Ala	Arg	Ser	Cys	Gly	Ser	Gln	Tyr	Phe	Asp	Thr	Trp	Gly	Gln	Gly	Ile	Leu	Val	Thr	Val	Ser			
Rabbit Daw	120	Pro	Ser	Gly	Thr	Lys	Ala	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	130	Thr	Leu	Gly	Cys	Leu	Val	Lys	Gly	Tyr	Leu											
	125	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	140	Thr	Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	Gly	Tyr	Leu								
Rabbit Daw	160	Pro	Glu	Pro	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Thr	Leu	Thr	Asp	170	Thr	Phe	Pro	Ser	Val	Arg	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Val	Pro	Ser					
	165	Pro	Glu	Pro	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Thr	Leu	Thr	Asp	Thr	180	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Val	Pro	Ser				
Rabbit Daw	190	Thr	Val	Ser	Val	—	—	—	—	—	—	—	—	—	—	—	200	Thr	Cys	Asx	Val	Ala	His	Ala	—	Thr	Asx	Thr	Lys	Val	Asp	Lys	Thr	Val	Ala	Pro	Ser	Thr
	200	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	210	Thr	Asn	Thr	Lys	Val	Asp	Lys	Thr	Val	Ala	Pro	Ser	Thr							
Rabbit Daw	230	Cys	Ser	Lys	Pro	—	Met	—	—	—	—	—	—	—	—	—	240	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Ile	Phe	Lys	Pro	Pro	Lys	Asp	Thr	Leu	Met	
	250	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met			

Fig. 6. Comparison of the sequences for residues 85–253 of the γ -chains of rabbit IgG with that of the γ -chain of a pathological human $\gamma 1$ IgG Daw (Press & Hogg, 1969). Identical residues are underlined, assuming deletions to give maximum homology, but as discussed in the text no satisfactory sequence could be established for residues 100–109. Numbering is as used in the Daw-protein sequence. The section represented by the major peptides discussed in the present paper are: peptide Ts4, residues 85–99; peptide T3, residues 100–170; peptide T7, residues 171–179; peptide T2, residues 180–253.

poor yield that their sequences could not be determined in full. It is likely that multiple sequences do occur and hence explain the failure to obtain peptides in good yield from the *N*-terminal part of peptide T3. Such evidence (Press & Hogg, 1969; Edelman *et al.* 1969) as is available from the γ -chain of myeloma proteins suggests that this may be an exceptionally variable area, and the homologous area in human κ -chains after the half-cystine residue in position 88 is highly variable (Milstein, 1969).

The evidence given above that peptide T4 is derived from the Fc fraction seems conclusive and leaves the section with residues 35–85 unaccounted for. Peptides that probably account for the sections with residues 35–50 and 65–84 have been isolated and their sequences determined, but no satisfactory overlaps have been obtained yet. There is no evidence that the sequence of residues 50–64 is highly complex and peptides probably derived from this section have also been isolated but there is the technical difficulty that, as judged by the sequences of the myeloma γ -chain, there are no easily characterized residues to guide the work in this area. Little progress has been made in characterizing the T1 fraction, which appears to be more complex than originally thought and to contain, in part, partial digest products of several sections of C-1 fraction.

In summary, the predominant sequence of *N*-terminal section of the rabbit γ -chain has been completed for residues 109–253 (Daw-protein numbering) and evidence has been obtained suggesting that much variability may occur between residues 100 and 109. Other work has given the sequences for residues 1–34 and 85–99 and it appears likely that much of the gap 35–84 can be completed. The small region of apparently high variability (residues 100–109) makes it likely that this may be an important section in determining the combining specificity, and if this is correct then a less complex picture from residues 100–109 might be expected when the γ -chain of a purified antibody is studied. However, there may well be many ways of arriving at combining sites of similar specificity and, if so,

no distinctive sequences in this or any other area that are characteristic of a given combining specificity would be found. An answer to this question should be obtainable from comparative sequence studies with purified antibodies. Singer, Slobin, Thorpe & Fenton (1968) reported that, after affinity labelling, they obtained from the heavy chain a dipeptide with threonine on the *N*-terminal side of the substituted tyrosine residue. There is a Thr-Tyr sequence in position 94–95, again drawing attention to the region near residues 100–110. An extension of affinity-labelling studies, if coupled with a complete major sequence, should give an answer as to the section of peptide chain forming the combining site.

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