The Amino Acid Sequences of the Fd Fragments of Two Human γ 1 Heavy Chains

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The amino acid sequences of the Fd fragments of two human pathological immunoglobulins of the immunoglobulin G1 class are reported. Comparison of the two sequences shows that the heavy-chain variable regions are similar in length to those of the light chains. The existence of heavy chain variable region subgroups is also deduced, from a comparison of these two sequences with those of another γ 1 chain, Eu, a μ chain, Ou, and the partial sequence of a fourth γ 1 chain, Ste. Carbohydrate has been found to be linked to an aspartic acid residue in the variable region of one of the γ 1 chains, Cor.

The chemical complexity of the immunoglobulins is in part related to antibody specificity, but some of the complexity can be ascribed to the many classes and subclasses into which the light and heavy chains can be grouped. Amino acid-sequence studies on light chains have shown that the C-terminal half of all light chains of a single class and allotype have an identical sequence, but that the sequence of the N-terminal half of the chain varies from one light chain to another. On the basis of 'fingerprint' studies heavy chains were also assumed to have constant and variable regions, each class and subclass being characterized by a different constant-region sequence. Antibody specificity is believed to be related to the variable part of the chains, and, in order to discover the extent of the variable region of the heavy chain, the amino acid sequences of Fd fragments of two pathological immunoglobulins of γ 1 subclass have been determined. The sequence of the N-terminal 84 residues of one heavy chain, Daw, has been reported previously (Piggot & Press, 1967; Press, 1967), as has the mid-chain sequence 211-253 (Steiner & Porter, 1967). Preliminary data on the chemical structure of the other heavy chain, Cor, have also been given (Press & Piggot, 1967). In this paper we describe the sequence studies on Daw heavy chain for residues 85-225, and on Cor heavy chain for residues 1-225. A report of some of these results was published by Press & Hogg (1969).

MATERIALS

Enzymes. The enzymes used were described by Press, Piggot & Porter (1966).

Other materials. Dithiothreitol was obtained from Cal-

biochem, Los Angeles, Calif., U.S.A. Iodo[1- 14 C]acetamide was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The other reagents were as described by Piggot & Press (1967).

Myeloma IgG (Cor).* This was isolated by precipitation from the plasma with Na₂SO₄ (18%) and purified by fractionation on a column of DEAE-Sephadex A-50 by using a 17-200mm concentration gradient of sodium phosphate buffer, pH 6.2.

Pathological IgG (Daw). This was prepared as described by Press *et al.* (1966).

METHODS

'Cor' Fab fragment. IgG (Cor) was digested with papain and fractionated on a column of Sephadex G-100 as described by Press *et al.* (1966). The Fab and Fc fragments were separated by chromatography on DEAE-cellulose at pH 8 as described by Deutsch, Thorpe & Fudenberg (1963).

'Cor' heavy chain. This was prepared by reduction of IgG with 5mm-dithiothreitol, alkylation with iodoacetamide and separation of the heavy chains from the light chains on a column of Sephadex G-100 in M-acetic acid.

'Daw' Fd fragment 2a'. Daw IgG was digested with papain and the Fab and Fc fragments were separated from undigested IgG on a column of Sephadex G-100 as described by Press *et al.* (1966). The mixture of Fab and Fc fragments was then digested with cyanogen bromide and chromatographed on a column of Sephadex G-100 in 6M-urea-0.2M-sodium formate, pH 3.3. The first peak to be eluted was the Fab fragment (the constituent cyanogen bromide cleavage products are disulphide bonded; Piggot & Press, 1967). This fraction was then totally reduced and alkylated with iodo[1- 14 C]acetamide

*Abbreviations: IgG, immunoglobulin G; PCA, Cmc, Hsr and CHO (in sequences and tables), pyrrolid-2-one-5-carboxylic acid, *S*-carboxyamidomethylcysteine, homoserine and carbohydrate respectively.

as described by Cebra, Givol & Porter (1968) and re-fractionated on the same column. The light chain, which contains no methionine residues and was therefore not cleaved by cyanogen bromide, was eluted first, followed by fragments 2a', 4 and 2b (see Piggot & Press, 1967).

Cyanogen bromide digestion. This was as described by Press *et al.* (1966).

C-Terminal analysis. C-Terminal amino acids of peptides were determined by digestion of 0.02 μ mol of peptide with carboxypeptidase A for various times and by hydrazinolysis as described by Press *et al.* (1966).

N-Terminal analysis. N-Terminal amino acids of peptides and fragments were determined by either the fluorodinitrobenzene method of Sanger (Porter, 1957) or by the 'dansyl' method of Gray (1967).

'Dansyl'-Edman technique. This was used, as described by Gray (1967), to determine the amino acid sequences of peptides. The acid and amide forms of aspartic and glutamic residues were distinguished by determining the mobility at pH 6.5 of the peptides containing these residues (Offord, 1966).

Enzymic digestion. This was done in 50 mM-NH₄HCO₃ at pH 8.1-8.3, with a peptide concentration of 3-5 mg/ml.

Measurement of radioactivity of ¹⁴C-labelled peptides. Aqueous samples were dissolved in the scintillation fluid described by Kinaird (1957), or were adsorbed on glass-fibre paper (Whatman G F/A) and placed in a scintillation fluid consisting of 0.6 g of 2,5-diphenyloxazole (PPO) and 12 mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (dimethyl-POPOP) in 100 ml of toluene and the radioactivity was measured in a Nuclear-Chicago liquid-scintillation counter.

RESULTS

Sequence of the Fd fragment of IgG (Cor)

The Fab fragment was cleaved with cyanogen bromide, totally reduced and alkylated with iodo-[1-¹⁴C]acetamide as described by Cebra *et al.* (1968) and fractionated on a column of Sephadex G-100 in 6M-urea-0.2M-sodium formate, pH 3.3. Five fractions were obtained: the first was whole light chain (there are no methionine residues in Cor light chain); the other four fragments 2a', 5a, 5b and 6a are derived from the Fd fragment (Press & Piggot, 1967). The amino acid composition of each of these fragments was determined (Tables 1, 2, 5 and 6) and their alignment in the Fd fragment was deduced by comparison with the amino acid sequence of Daw Fd fragment (Fig. 10).

Fragment 5b. This was digested with chymotrypsin (80 μ g/ μ mol at 37°C for 3h) and the digest was fractionated by gel filtration on a column of Sephadex G-25 in 0.02M-ammonia and paper electrophoresis at pH 1.9. Five peptides were isolated that accounted for the composition of fragment 5b (see Table 1), and the amino acid sequences of these peptides were determined as shown in Fig. 1. Fragment 5b had been assumed to be the N-terminal fragment of the heavy chain, because of its very similar amino acid composition

Table 1. Amino acid composition and isolation procedures of the chymotryptic peptides of Cor fragment 5b

Amino acid	Composition (mol of amino acid/mol of peptide)						Fragment 5b
	Peptide C2.a	Peptide C2.b	Peptide C3.a	Peptide C3.c	Peptide C4.b	Sum of peptides	
Lys	0	0.9	0	0	0	1	1.0
Arg	0	1.0	0	0	0	1	1.0
Thr	1.0	1.6	0.9	2.9	0	7	6.6
Ser	3.0	1.3	0	0	1.0	5	5.1
Glu	0	2.1	1.1	0	0	3	3.6
Pro	0	1.8	0	0	0	2	2.0
Gly	1.1	1.3	0	0	1.1	3	3.2
Ala	0	1.2	0	0	0	1	1.4
Val	0	0.9	1.0	0	0	2	2.0
Leu	1.0	1.9	1.0	1.2	0	5	4.7
Phe	0	0	0	1.2	0.9	2	1.8
Cmc	0	0	0	0.7	0	1	0.9
Hsr	0.9	0	0	0	0	1	0.7
Total	7	14	4	6	3	34	34
G-25 elution volume*	1.2	1.2	1.4	1.4	1.6		
Mobility at pH 1.9†	+0.35	+0.53	+0.10	+0.40	+0.50		

* Relative to exclusion = 1.0.

† Mobility relative to lysine = +1.0.

Table 3. *Chymotryptic peptides of Cor fragment 5a*

The tryptophan content was assumed to be 1 for those peptides that stained with the Ehrlich reagent on paper. Hexosamine was calculated from the amino acid analyser trace, without correction for recovery.

Amino acid	Composition (mol of amino acid/mol of peptide)						
	Peptide C2.c	Peptide C3.c	Peptide C5.a	Peptide C5.d	Peptide C6.a2	Peptide C6.b	Peptide C7.e(C7.b)
Lys	1.1	0	1.0	0	1.2	0	0
Arg	0	0.9	1.2	0	0.7	0.8	0
Asp	3.2	1.1	2.1	0	0	1.3	0
Thr	0	1.8	1.8	1.1	0	0	0
Ser	0	1.1	2.2	0	0	0	0
Glu	0	1.1	1.3	0	1.8	0	0
Pro	0	0	0	0	2.2	0	0
Gly	0	0	0	0	2.2	0	1.2
Ala	0	0	0	0	0	1.1	0
Val	0	0	1.4	0	0	0	1.1
Ile	0	0	1.0	0	0.9	0.9	0
Leu	0	2.0	1.0	0	1.0	0.9	0
Tyr	1.7	0	0	0	0	0	0
Cmc	0	0	0	0	0	0	0.7
Hsr	0	0	0	0.9	0	0	0
Trp	0	0	0	0	1	1	1
Total	6	8	13	2	11	6	4
Hexosamine		1.0					

T1.a and T1.c; it was also detected in the chymotryptic peptide C3.c (see below). Fragment 5a was digested with chymotrypsin (200 $\mu\text{g}/\mu\text{mol}$ at 37°C for 3h) and the digest was fractionated. The compositions of the chymotryptic peptides are given in Table 3 and the methods used in the isolation of the tryptic and chymotryptic peptides are shown in Table 4. The sequence of fragment 5a was determined, as shown in Fig. 2. Tryptic peptide T4.a was digested with chymotrypsin and the two peptides T4.a.C1 and T4.a.C2 were purified by paper electrophoresis at pH 3.5. Chymotryptic peptides C7.b and C7.e had the same composition, but peptide C7.e is acidic and its α -amino group is blocked. Tryptic peptide T5.a also has a blocked α -amino group, and the *N*-terminal residue of fragment 5a could not be detected by either the 'dansyl' method or the method of Edman degradation. A blocked *N*-terminal alkylated cysteine residue had previously been found in Daw fragment 4 (Press, 1967) and presumably arises as a result of condensation of the α -amino group with the carboxamidomethyl side chain. Peptides T2.b1 and T2.b5 were neutral at pH 6.5. Peptide T3.c was basic and peptide T4.a.C2 was acidic. Peptide T1.b was acidic with a mobility of -0.7, from which it was deduced that the four aspartic residues were present as aspartic acid and this was confirmed by the mobilities of peptides C6.b (neutral) and C2.c (-0.65). Peptide C3.c, which contained hexosamine, was neutral at pH 6.5. As the carbohydrate was

shown to be attached to the aspartic residue of this peptide, presumably involving the β -carboxyl group, and as sialic acid is unlikely to be present on the peptide (see section below on carbohydrate of Cor Fd fragment), the glutamic residue in peptide C3.c is probably present in the acid form. The sequence of fragment 5a is complete as shown in Fig. 2.

Fragment 6a. This was digested with trypsin (100 $\mu\text{g}/\mu\text{mol}$ at 37°C for 2h) and two peptides, 6a.T1 and 6a.T2, were isolated from the digest. Peptide 6a.T1 was further digested with chymotrypsin, and three peptides were separated by gel filtration. The amino acid composition and isolation procedures of these peptides are given in Table 5, together with the amino acid composition of fragment 6a. The sequence of this fragment was determined, as shown in Fig. 3. The mobility of peptide 6a.T1 (see Table 5) established that both aspartic residues are present as aspartic acid.

Fragment 2a'. This has an *N*-terminal aspartic residue, and its composition is given in Table 6, which shows that it contains no homoserine and is therefore the *C*-terminal fragment of the Fd fragment. It was digested with trypsin (200 $\mu\text{g}/\mu\text{mol}$ at 37°C for 5h). A precipitate formed and this was separated by centrifugation and found to contain about half the total digest. The precipitate represents a large insoluble peptide, 2a'.T1. The soluble portion of the tryptic digest was fractionated on columns of Sephadex G-50 and G-25 and eight

Table 4. *Isolation procedures for the component peptides of Cor fragment 5a*

	Sephadex-gel filtration			Paper electrophoresis	
	Type	Solvent	Elution vol.*	pH or chromatography system	Mobility† or R_F
Tryptic peptides					
T1.a	(a) G-25	0.02 M-NH ₃	1.0		
	(b) G-50	0.05 M-NH ₃	1.2	6.5	-0.3
T1.b	(a) G-25	0.02 M-NH ₃	1.0		
	(b) G-50	0.05 M-NH ₃	1.3	6.5	-0.7
T1.c	(a) G-25	0.02 M-NH ₃	1.0		
	(b) G-50	0.05 M-NH ₃	1.5	6.5	0
T2.bl	G-25	0.02 M-NH ₃	1.2	3.5	+0.1
				BAW‡	0.8
T2.b5	G-25	0.02 M-NH ₃	1.2	3.5	+0.1
				BAW‡	0.1
T2.C4	G-25	0.02 M-NH ₃	1.2	3.5	0
				BAW‡	0.2
T3.a	G-25	0.02 M-NH ₃	1.4	BAW‡	0.5
T3.c	G-25	0.02 M-NH ₃	1.4	BAW‡	0.1
T4.a	G-25	0.02 M-NH ₃	1.8	3.5	+0.2
T5.a	G-25	0.02 M-NH ₃	1.9	3.5	0
Chymotryptic peptides					
C2.c	G-50	0.05 M-NH ₃	1.5	3.5	0
C3.c	G-50	0.05 M-NH ₃	1.7	3.5	+0.1
C5.a	G-50	0.05 M-NH ₃	1.9	3.5	+0.6
C5.d	G-50	0.05 M-NH ₃	1.9	3.5	+0.2
C6.a2	G-50	0.05 M-NH ₃	2.2	3.5	+0.5
				1.9	+0.6
C6.b	G-50	0.05 M-NH ₃	2.2	3.5	+0.3
				6.5	0
C7.b	G-50	0.05 M-NH ₃	2.8	3.5	0
C7.e	G-50	0.05 M-NH ₃	2.8	3.5	-0.6

* Relative to exclusion volume = 1.0.

† Mobility relative to lysine = +1.0 and to aspartic acid -1.0.

‡ Chromatography in butan-1-ol-acetic acid-water (12:3:5, by vol.).

tryptic peptides were isolated as shown in Table 7. The composition of the nine tryptic peptides given in Table 6 accounts for the composition of the whole of fragment 2a'.

Peptide 2a'.T1 had aspartic acid as the *N*-terminal residue and it was digested with chymotrypsin (570 µg/µmol at 37°C for 2½ h). The soluble digest was fractionated on a column of Sephadex G-50 in 0.05 M-ammonia. Nine peptides were isolated, which together accounted for the composition of 2a'.T1. (see Tables 8 and 7 for composition and methods of purification of these peptides). The amino acid sequences of the peptides were determined as shown in Fig. 4. Peptide T1.C2a was shown to be the *N*-terminal peptide of peptide T1 by isolation of a peptide 2a'.C3.6a from a chymotryptic digest of peptide 2a' (see next section and Fig. 7). Peptide T1.C6h is the only lysine-containing peptide, and must therefore be *C*-terminal. Peptides T1.C4e and T1.C4b were overlapped by another peptide, C3.5d, isolated from the chymotryptic digest of peptide 2a' (see Fig. 7). Peptide T1.C5a4

was an extension of peptide T1.C6d and overlapped with peptide T1.C6h. Peptide T1.C6g is the *C*-terminal tripeptide of peptide T1 and its amino acid sequence was determined as shown in Fig. 4. The other chymotryptic peptides of peptide T1 were aligned in the order shown in Fig. 4, by the similarity in sequence between this human heavy chain and rabbit heavy chain in this region (see Fig. 5) (Fruchter, Jackson, Mole & Porter, 1970). The full stops in the sequence of Cor peptide 2a'.T1 indicate where overlapping peptides were not available and the sequence was deduced by homology with the rabbit heavy chain. The aspartic and glutamic residues in peptide T1.C2a must be present in the acid form, since the chymotryptic peptide 2a'.C3.6a referred to above had a mobility of -0.13 at pH 6.5. All the other aspartic and glutamic residues of peptide T1 are in the amide form, since peptides T1.C5b2, T1.C4d, T1.C4b and T1.C5a4 are neutral at pH 6.5 and peptide T1.C6g is basic.

The method of sequence determination of the

Fig. 2. Amino acid sequence of Cor fragment 5a. Sequence determination from the *N*-terminal end was by the 'dansyl'-Edman technique (→) and from the *C*-terminal end by carboxypeptidase A digestion (←) or by hydrazinolysis (↔).

Table 5. Amino acid composition and isolation procedures of Cor fragment 6a and tryptic and chymotryptic peptides derived from it

Amino acid	Composition (mol of amino acid residue/mol of peptide)					Fragment 6a
	Peptide 6aT1	Peptide 6aT2	Peptide 6aT1.C1	Peptide 6aT1.C2	Peptide 6a.T1.C3	
Arg	0.9	0	0	0.9	1.0	0.8
Asp	2.0	0	2.2	0	0	2.0
Thr	2.0	1.3	2.0	0	0	3.5
Pro	1.3	1.7	0.9	0	0	3.2
Gly	0.6	1.5	0	0	0	1.5
Ala	1.9	2.2	1.1	1.1	1.2	3.8
Val	1.1	0.8	0.9	0	0	1.9
Ile	0	1.7	0	0	0	1.9
Tyr	1.8	0.9	1.0	0	0.8	2.6
Cmc	1	0	0	1	1	0.7
Hsr	0	0.9	0	0	0	1.1
Total Residues	12	11	8	3	4	23
Sephadex G-25 0.02M-NH ₃ *	1.1	1.2	1.2	1.8	2.0	—
Mobility at pH 6.5†	-0.2	0	—	—	—	—

* Elution volume given relative to exclusion volume.

† Mobility relative to aspartic acid = -1.0.

Fig. 3. Amino acid sequence of Cor fragment 6a. Sequence determination from the *N*-terminal end was by the 'dansyl'-Edman technique (→) and from the *C*-terminal end by carboxypeptidase digestion (←).

Table 6. *Tryptic peptides of Cor fragment 2a'*

The *S*-carboxyamidomethylcysteine composition of fragment 2a' is calculated from amino acid analysis. *S*-Carboxyamidomethylcysteine residues are based on the radioactivity of the peptides. The tryptophan composition of fragment 2a' is calculated from the extinction at 280 nm. The number of tryptophan residues in the peptides was assumed to be 1 when the peptide stained with Ehrlich reagent on paper. The composition of fragment 2a' is based on 6 residues of alanine/molecule of fragment 2a'. The composition of peptide T1 is taken from the sum of the constituent residues of its chymotryptic peptides (Table 8, column 10).

Amino acid	Composition (mol of amino acid residue/mol of peptide)										Fragment 2a'
	Peptide T1	Peptide T4.1c	Peptide T4.1d	Peptide T4.2a	Peptide T4.2b	Peptide T4.2d	Peptide T4.2e	Peptide T4.3f	Peptide T5	Sum of peptides	
Lys	2	1.0	0.9	1.1	1.0	1.0	1.7	0	0	9	7.9
His	2	0	0	0	0	0	0	0.9	0	3	2.4
Arg	0	0	0	0	0	0	0	0	0.9	1	1.1
Asp	5	0	0	1.0	1.1	0	0	0	1.1	8	8.4
Thr	7	2.1	2.6	0	0	0	0	1.1	0	13	13
Ser	11	2.4	3.1	0.9	0	3.1	0	0	0	20	20
Glu	3	0	0	0	0	0	1.0	0	0	4	4.3
Pro	5	0	1.0	0	0	2.8	1.2	0	0	10	10
Gly	4	2.9	1.0	0	0	1.2	0	0	1.2	10	9.6
Ala	2	1.9	1.1	0	0	1.0	0	0	0	6	6.0
Val	8	1.0	1.9	0	0.9	1.1	0.9	0	0.8	15	13
Ile	1	0	0	0	0	0	0	0	0	1	1.2
Leu	5	1.8	0	0	0	1.0	0	0	0	8	7.9
Tyr	3	0	0	0	0	0	0	0	0	3	3.0
Phe	2	0	0	0	0	0.9	0	0	0	3	2.9
Cmc	1	1	0	1	0	0	0	0	0	3	2.0
Trp	1	0	0	0	0	0	0	0	1	2	2.0
Total residues	62	14	12	4	3	12	5	2	5	119	115

eight smaller tryptic peptides of fragment 2a' is shown in Fig. 6. Peptides T4.1c, T4.1d and T5 were digested with chymotrypsin and the amino acid sequences of the chymotryptic peptides determined as shown. The mobilities of the four peptides containing glutamic and aspartic residues are also

given in Fig. 6, and establish that these residues are all present in the acid form.

The tryptic peptides of peptide 2a' were aligned by isolation and partial sequence-determination of nine chymotryptic peptides. Fragment 2a' was digested with chymotrypsin (500 μg/μmol at 37°C

Table 7. *Isolation procedures for component peptides of Cor fragment 2a'*

	Sephadex-gel filtration			Paper electrophoresis	
	Type	Solvent	Elution volume*	pH or chromatography system	Mobility† or R_F
Tryptic peptides					
T4.1c	G-50	0.05 M-NH ₃	1.8		
	G-25	0.02 M-NH ₃	1.0	6.5	+0.2
T4.1d	G-50	0.05 M-NH ₃	1.8		
	G-25	0.02 M-NH ₃	1.0	6.5	+0.3
T4.2a	G-50	0.05 M-NH ₃	1.8		
	G-25	0.02 M-NH ₃	1.2	6.5	-0.04
T4.2b	G-50	0.05 M-NH ₃	1.8		
	G-25	0.02 M-NH ₃	1.2	6.5	0
T4.2d	G-50	0.05 M-NH ₃	1.8	3.5	+0.5
	G-25	0.02 M-NH ₃	1.2	6.5	+0.25
T4.2e	G-50	0.05 M-NH ₃	1.8	PAW‡	0.35
	G-25	0.02 M-NH ₃	1.2	6.5	+0.4
T4.3f	G-50	0.05 M-NH ₃	1.8		
	G-25	0.02 M-NH ₃	1.3	6.5	+0.5
T5	G-50	0.05 M-NH ₃	2.5	—	—
Chymotryptic peptides of peptide T1					
T1.C2a	G-50	0.05 M-NH ₃	1.3	3.5	-0.6
T1.C4b	G-50	0.05 M-NH ₃	1.7	BAW‡	0.3
T1.C4d	G-50	0.05 M-NH ₃	1.7	BAW‡	0.45
T1.C4e	G-50	0.05 M-NH ₃	1.7	BAW‡	0.65
T1.C5a4	G-50	0.05 M-NH ₃	1.9	3.5	+0.05
				BAW‡	0.4
T1.C5b1	G-50	0.05 M-NH ₃	1.9	3.5	+0.2
T1.C5b2	G-50	0.05 M-NH ₃	1.9	3.5	+0.25
				BAW‡	0.5
T1.C5e	G-50	0.05 M-NH ₃	1.9	3.5	+0.5
T1.C6d	G-50	0.05 M-NH ₃	2.3	3.5	+0.2
T1.C6g	G-50	0.05 M-NH ₃	2.3	3.5	+0.7
T1.C6h	G-50	0.05 M-NH ₃	2.3	3.5	+0.8
Chymotryptic peptides					
C3.5d	G-50	0.05 M-NH ₃	1.8	3.5	-0.1
				BAW‡	0.5
C3.6a	G-50	0.05 M-NH ₃	1.8	3.5	0
				6.5	-0.1
C3.6b.c	G-50	0.05 M-NH ₃	1.8	3.5	0
				6.5	-0.05
C3.7d	G-50	0.05 M-NH ₃	1.8	BAW‡	0.35
				3.5	+0.1
C3.11	G-50	0.05 M-NH ₃	1.8	6.5	+0.1
				3.5	+0.8
C4.2.3c	G-50	0.05 M-NH ₃	2.0		
	G-25	0.02 M-NH ₃	1.2	3.5	+0.6
C4.2.9b	G-50	0.05 M-NH ₃	2.0	6.5	+0.3
	G-25	0.02 M-NH ₃	1.2	3.5	0
C4.3f5	G-50	0.05 M-NH ₃	2.0	BAW‡	0.2
	G-25	0.02 M-NH ₃	1.4	3.5	+0.45
C5.1	G-50	0.05 M-NH ₃	2.2	6.5	+0.30
				3.5	-0.5

* Relative to exclusion volume = 1.0.

† Mobility relative to lysine = +1.0 and to aspartic acid = -1.0.

‡ Chromatography in pyridine-3-methylbutan-1-ol-water (7:7:6, by vol.) (PAW) or in butan-1-ol-acetic acid-water (12:3:5, by vol.) (BAW).

Table 8. *Chymotryptic peptides of Cor tryptic peptide 2a'T1*

The composition of peptide T1 is based on 2 alanine residues/molecule of peptide. The *S*-carboxyamido-methylcysteine composition of peptide T1 is calculated from the amino acid analysis. *S*-Carboxyamidomethylcysteine residues are based on the radioactivity of the peptides.

Amino acid	Composition (mol of amino acid residue/mol of peptide)									Sum of peptides	Peptide T1
	Peptide T1C2a	Peptide T1C4b	Peptide T1C4d	Peptide T1C4e	Peptide T1C5b1	Peptide T1C5b2	Peptide T1C5e	Peptide T1C6d	Peptide T1C6h		
Lys	0	0	0	0	0	0	0	0	2.0	2	1.9
His	0	0	0	0	0	0	0.8	0	0.9	2	1.4
Arg	0	0	0	0	0	0	0	0	0	0	0.1
Asp	1.1	0	0	0	0	0.8	0	1.1	1.9	5	4.1
Thr	1.0	1.9	0	1.0	1.0	0	1.0	0	1.0	7	6.3
Ser	1.1	0	2.0	5.5	0	1.2	1.2	0	1.1	11	10.0
Glu	1.0	1.0	1.0	0	0	0	0	0	0	3	2.9
Pro	1.9	0	0	1.1	1.0	0	0	0	1.1	5	4.2
Gly	0	1.2	1.2	0	0	1.2	1.2	0	0	4	4.0
Ala	0	0	0	0	1.0	1.0	0	0	0	2	2.0
Val	1.9	0	0	2.5	1.0	0	0.8	0	1.0	8	6.3
Ile	0	0	0	0	0	0	0	0.9	0	1	0.9
Leu	0	0	1.0	1.9	1.2	0.8	0	0	0	5	4.7
Tyr	1.0	0.9	0.8	0	0	0	0	0	0	3	2.6
Phe	1.0	0	0	0	0.8	0	0	0	0	2	2.0
Cmc	0	0	0	0	0	0	0	1.0	0	1	0.7
Trp	1.0	0	0	0	0	0	0	0	0	1	1.0
Total residues	11	5	6	12	6	5	5	3	9	62	55

for 2½ h) and the digest was fractionated on a column of Sephadex G-50 in 0.05 M-ammonia. The methods of purification of the relevant peptides are shown in Table 7 and their compositions are given in Table 9. Fig. 7 shows the partial-sequence data on the chymotryptic peptides and the consequent alignment of the tryptic peptides. The *N*-terminal residue of fragment 2a' is aspartic acid, which is in agreement with the sequence shown in Fig. 7. A chymotryptic peptide overlapping tryptic peptides T4.2d and T4.1c was not found, but two peptides, C4.2.9b and C3.6bc, were isolated and, as shown in Fig. 7, these peptides come from this part of the sequence. The latter peptide results from cleavage by chymotrypsin (or contaminating trypsin) of the Lys-Ser bond. The chymotryptic peptide C4.2.3c must occur at the *C*-terminal end of fragment 2a', as this is the only position in the sequence where it can be placed. Papain presumably digested the Cor heavy chain at this histidine residue. Steiner & Porter (1967) reported that papain digested Daw heavy chain at a histidine residue in an identical sequence consisting of peptides C3.11-C4.2.3c. Peptides derived from the tryptic peptide T1 were also found in the chymotryptic digest and one of these, C3.5d, overlapped two of the peptides derived from the chymotryptic digest of peptide T1 (T1.C4e and T1.C4b). The sequence of Cor fragment 2a' is therefore almost

complete, as shown in Fig. 7, except for the order of the five chymotryptic peptides of peptide T1, which have been aligned by comparison with the rabbit heavy chain, as described above.

Alignment of the four fragments of Cor Fd fragment. Fragment 5b has been shown to be the *N*-terminal fragment of the Fd fragment and fragment 2a' the *C*-terminal fragment. Fragment 5a is assumed to follow fragment 5b, and fragment 6a to be the third fragment from the *N*-terminal end, by the similarity in sequence of Cor fragment 5a to Daw fragment 4 (Press, 1967) and the similarity of Cor fragment 6a to the *N*-terminal sequence of Daw fragment 2a' (see next section). The complete sequences of Daw and Cor Fd fragments are compared in Fig. 10.

Sequence of Daw fragment 2a'

The amino acid sequence of the first 84 residues of Daw heavy chain has already been determined (Piggot & Press, 1967; Press, 1967). The fragment 2a' comprised the *C*-terminal part of Daw Fd fragment from residue 85 onwards. This fragment has an *N*-terminal asparagine residue (see mobility of peptide T2.5 given below) and its sequence has been established by isolating the tryptic and chymotryptic peptides. The sequence becomes identical with that of Cor heavy chain from residue

trypsin (400 $\mu\text{g}/\mu\text{mol}$ at 37°C for 4h). The digest was centrifuged, and the precipitate contained more than half the total digest and was found to be a large insoluble peptide T1 of 62 residues (Table 10). This peptide had a composition identical with that of Cor peptide 2a'. T1, and its amino acid sequence was established by isolating the constituent chymotryptic peptides, and found to be

Fig. 6. Amino acid sequence of the tryptic peptides of Cor 2a' (except peptide T1, shown in Fig. 4). Sequence determination from the *N*-terminal end was by the 'dansyl'-Edman technique (→) and from the *C*-terminal end by carboxypeptidase A digestion (←) or hydrazinolysis (←+).

Table 9. Chymotryptic peptides of Cor fragment 2a'

The *S*-carboxyamidomethylcysteine residues are calculated from the radioactivity of the peptides. The tryptophan residues are assumed to be 1 when the peptide stained with Ehrlich's reagent.

Amino acid	Composition (mol of amino acid residue/mol of peptide)								
	Peptide C3.5d	Peptide C3.6a	Peptide C3.6bc	Peptide C3.7d	Peptide C3.11	Peptide C4.2.3c	Peptide C4.2.9b	Peptide C4.3f5	Peptide C5.1
Lys	0	0.8	0	0.8	3.3	1.0	0	0	0
His	0	0	0	0	0	0.9	0	0	0
Arg	0	0	0	0	0	0	0	0.8	0
Asp	0	1.2	0	0	1.2	1.0	0	0	1.0
Thr	2.2	1.1	1.6	1.0	0	0.9	0	1.8	0
Ser	5.8	1.7	2.0	3.8	0	1.2	2.1	0	0
Glu	0.9	0.9	0	0	1.1	0	0	0	0
Pro	1.4	2.0	0	2.2	1.1	0	0.9	1.2	0
Gly	1.7	0.9	2.3	1.3	0	0	0	2.4	0
Ala	0	0	2.2	1.4	0	0	1.0	0	0
Val	2.2	2.7	0	1.0	2.2	0	0	1.8	1.0
Ile	0	0	0	0	0	0	0	0	0
Leu	2.1	0.8	1.1	0.8	0	0	0	0	0
Tyr	0.7	0.8	0	0	0	0	0	0	0
Phe	0	0.9	0	0.7	0	0	0	0	0
Cmc	0	1	0	0	0	1	0	0	0
Trp	0	1	0	0	0	0	0	0	1
Total residues	17	16	9	13	9	6	4	8	3

Fig. 7. Amino acid sequence of Cor fragment 2a', showing partial-sequence data on the chymotryptic peptides and the alignment of all the tryptic peptides. The sequence determination was by the 'dansyl'-Edman technique (→) and by carboxypeptidase A digestion (←). Peptides C3.7d.T1 and C3.7d.T2 were tryptic peptides of peptide C3.7d; peptides C3.6a.T1 and C3.6a.T2 were tryptic peptides of peptide C3.6a.

identical with that of Cor peptide 2a'.T1. The soluble portion of the tryptic digest was fractionated by gel filtration and paper electrophoresis and the method of isolation and the compositions of the relevant tryptic peptides are shown in Tables 11 and 10 respectively. Seven of the nine tryptic peptides were identical with seven of the tryptic peptides isolated for Cor fragment 2a' (Table 6); their amino acid sequences were determined and no differences were detected. The amino acid sequences of the other two peptides, T2.5 and T2.p, were determined as shown in Fig. 8.

Peptide T2.5. This was digested with chymotrypsin. The isolation and compositions of the chymotryptic peptides, T2.5.C1, T2.5.C2, and T2.5.C3, are shown in Table 12 and their sequence is given in Fig. 8. Peptide T2.5 was neutral at pH 6.5 and after one step of the Edman degradation the residual 'peptide' was also neutral; therefore the *N*-terminal residue must be asparagine and the other aspartic residue is present as aspartic acid.

Peptide T2.p. This had a very low solubility, and

it was found impossible to purify it by paper electrophoresis since it could not be eluted from paper after electrophoresis in more than 10% yield. It was, however, possible to precipitate it from the gel-filtration fraction concentrated to 0.5 μmol of peptide/ml by adding acetic to a concentration of 1M and leaving at 2°C overnight. The precipitate was centrifuged and washed with 0.1M-acetic acid at 0°C, and on analysis was found to be pure (see Table 10). It was recovered in 60% yield by this procedure, and peptide T2.5 was isolated from the supernatant by electrophoresis (Table 11). Peptide T2.p has serine as the *N*-terminal residue. It was digested with chymotrypsin (800 μg/μmol at 37°C for 4h) and six peptides were isolated from the digest by paper electrophoresis at pH 3.5 (see Table 12). The amino acid sequences of these chymotryptic peptides of peptide T2.p were determined as shown in Fig. 8.

Peptide T2.p.C3 must be the *N*-terminal peptide, since it has serine as the *N*-terminal residue. Peptide T2.p.C4 contains lysine and must therefore be the

Table 10. *Tryptic peptides of Daw fragment 2a'*

The *S*-carboxyamidomethylcysteine composition of fragment 2a' is calculated from the amino acid analysis. The *S*-carboxyamidomethylcysteine residues in the tryptic peptides are calculated from the radioactivity of the peptides. The tryptophan content in fragment 2a' is calculated from the extinction at 280 nm. The tryptophan content of the peptides is based on staining of the peptides on paper with Ehrlich's reagent. The composition of fragment 2a' is based on 8 alanine residues/molecule of fragment 2a'. The composition of peptide T1 is based on the sum of the residues of its constituent chymotryptic peptides (Table 8). The numbers of the equivalent Cor 2a' tryptic peptides are given in parentheses under the numbers of the appropriate Daw tryptic peptides.

Amino acid	Composition (mol of amino acid/mol of peptide)									Sum of peptides	Fragment 2a'
	Peptide T1 (T1)	Peptide T2.5	Peptide T2.p	Peptide T3.1 (T4.2a)	Peptide T3.2 (T4.2b)	Peptide T3.3 (T4.1c)	Peptide T3.4 (T4.2d)	Peptide T3.6 (T4.2e)	Peptide T.4 (T4.3f)		
Lys	2	0	1.1	1.0	1.1	1.1	1.2	2.1	0	9	8.0
His	2	0	0	0	0	0	0	0	1.0	3	2.4
Arg	0	1.1	0	0	0	0	0	0	0	1	0.8
Asp	5	1.8	1.3	1.0	1.0	0	0	0	0	10	10.0
Thr	7	2.4	1.9	0	0	1.9	0	0	1.0	15	14.0
Ser	11	0	5.1	1.0	0	2.2	3.2	0	0	22	21.0
Glu	3	0	2.0	0	0	0	0	1.2	0	6	6.0
Pro	5	1.3	0	0	0	0	2.4	0.8	0	10	10.0
Gly	4	2.2	2.9	0	0	3.2	1.2	0	0	13	13.0
Ala	2	2.2	1.3	0	0	1.8	1.1	0	0	8	8.0
Val	8	1.2	2.0	0	0.9	1.0	1.0	0.9	0	15	14.0
Ile	1	0	0.9	0	0	0	0	0	0	2	1.9
Leu	5	0	1.0	0	0	1.8	1.0	0	0	9	9.0
Tyr	3	1.8	1.6	0	0	0	0	0	0	7	6.0
Phe	2	0	0.9	0	0	0	0.9	0	0	4	3.7
Cmc	1	1	1	1	0	1	0	0	0	5	3.4
Trp	1	0	1	0	0	0	0	0	0	2	2.0
Total residues	62	15	24	4	3	14	12	5	2	141	133.2

C-terminal peptide. Peptide T2.p.C5 is a shorter part of peptide T2.p.C4. The three peptides, T2.p.C3, C1 and C4, together account for the composition of peptide T2.p. Peptide T2.p.C3 is neutral at pH 6.5 and peptides T2.p.C1 and T2.p.C1a both have one net negative charge, and therefore the glutamic residue in peptide T2.p.C1 must be glutamine and the aspartic must be present as aspartic acid. A peptide 2a'C6d, Phe-Asp-Tyr-Trp, overlapping peptides T2.p.C3 and T2.p.C1 was isolated from a chymotryptic digest of the whole of Daw fragment 2a' (see next section), thus confirming the sequence of peptide T2.p shown in Fig. 8.

Chymotryptic peptides. Fragment 2a' was digested with chymotrypsin (500 $\mu\text{g}/\mu\text{mol}$ at 37°C for 4½ h). The digest was fractionated on a column of Sephadex G-50 in 0.05 M-ammonia. The constituent chymotryptic peptides of the tryptic peptide T1 were isolated, together with the chymotryptic peptides overlapping the tryptic peptides that are common to Cor and Daw 2a' fragments. In addition the chymotryptic peptides derived from the *N*-terminal part of Daw fragment 2a', comprising the tryptic

peptides T2.5, T2.p and T3.4, were isolated by the procedures shown in Table 11 and their analyses are given in Table 13.

Peptide C3.a. This has the same sequence as the *N*-terminal 11 residues of tryptic peptide T2.5 (see Fig. 9). Peptide T2.5 must therefore be the *N*-terminal peptide of fragment 2a', which fragment has asparagine as *N*-terminal residue. This is further confirmed by the comparison of the sequences of Cor and Daw heavy chains (Fig. 10).

Peptide C5.g1. The partial amino acid sequence of this peptide was determined as shown in Fig. 8. Peptides C5.g1.Tc and Ta are tryptic peptides of peptide C5.g1. This peptide overlaps the two tryptic peptides, T2.5 and T2.p, of fragment 2a' (see Fig. 9).

Peptide C4.f. The partial amino acid sequence of this peptide was determined as shown in Fig. 8. It contains the *C*-terminal part of tryptic peptide T2.p and the *N*-terminal part of peptide T3.4 (which has a sequence identical with that of Cor peptide 2a'.T4.2d; see Fig. 6). Another chymotryptic peptide, C4.e, also overlaps the two tryptic peptides (see Fig. 9). Peptide C4.f has an identical

Table 11. *Isolation procedures for the tryptic peptides and some chymotryptic peptides of Daw fragment 2a'*

Tryptic peptides	Sephadex G-50 (0.05M-NH ₃) elution volume*	Paper electrophoresis	
		pH or chromatography	Mobility† <i>R_F</i>
T2.5	1.5	3.5	+0.3
T2.p	1.5	Precipitation (see the text)	-0.04
T3.1	2.0	6.5	0
T3.2	2.0	6.5	+0.2
T3.3	2.0	6.5	+0.3
T3.4	2.0	6.5	+0.4
T3.6	2.0	6.5	+0.5
T4	2.7	3.5	-0.3
Chymotryptic peptides			
C3.a	1.9	3.5	+0.45
C4.fd	2.1	PAW‡	0.4
C5.a	2.2	6.5	-0.25
C5.d9	2.2	6.5	0
		BAW‡	0.8
C5.e8	2.2	6.5	0
		BAW‡	0.7
C5.g1	2.2	6.5	+0.3
C6.c1	2.7	3.5	-0.2
		BAW‡	0.4
C6.c2	2.7	3.5	-0.2
		BAW‡	0.6
C6.d	2.7	3.5	-0.1
		BAW‡	0.8

* Relative to exclusion volume = 1.0.

† Mobility relative to lysine = +1.0, to aspartic acid -1.0.

‡ Chromatography in pyridine-3-methylbutan-1-ol-water (7:7:6, by vol.) (PAW) or in butan-1-ol-acetic acid-water (12:3:5, by vol.) (BAW).

counterpart, in Cor 2a' chymotryptic peptide C3.7d, and from this point the sequence of Daw fragment 2a' is identical with that of Cor fragment 2a'. The identity in sequence was established by determining the amino acid sequences of all the constituent tryptic and chymotryptic peptides of Daw fragment 2a' (N. M. Hogg, unpublished work; this work forms part of a Thesis to be presented by N. M. H. in partial fulfilment of the requirements for a Ph.D.).

Peptides C6.d, C5.e8, C5.d9 and C5.a. These were also isolated (see Tables 11 and 13) and Fig. 9 shows that these peptides are derived from tryptic peptide T2.p and confirm the sequence of the latter peptide. Free tyrosine and phenylalanine (C6.c1 and C6.c2) were also present in the chymotryptic digest of fragment 2a' and are presumably derived from this part of the sequence (see Fig. 9). The complete amino acid sequence of Daw fragment 2a' (residues 85-225) is shown in Fig. 10.

Intrachain disulphide bridges in Cor and Daw heavy chains

The heavy chain of Cor was digested with cyanogen bromide and fractionated on a column of

Sephadex G-100 in 6M-urea-0.2M-sodium formate as described by Piggot & Press (1967) for the cleavage products of Daw heavy chain. Fraction 5 (elution volume 2.5 relative to exclusion volume = 1.0) was totally reduced as described by Cebra *et al.* (1968) and alkylated with iodo[1-¹⁴C]acetamide and re-run on the same column of Sephadex G-100. The three fragments 5a, 5b and 6a, described above, were separated. Fragments 5b and 6a were radioactive, but fragment 5a was not radioactive although it contains an alkylated cysteine residue (see Fig. 2). Presumably the cysteine residue in fragment 5a was alkylated during the mild reduction and alkylation of the IgG before the isolation of the heavy chain. The cysteine residues in fragments 5b and 6a must be linked by a disulphide bond, and these cyanogen bromide fragments, joined by a disulphide bond, were eluted in the same elution volume as fragment 5a on the first column. The two cysteine residues in Cor fragment 2a', which occur in peptides T4.1c and T1.C6d (Figs. 4 and 7), are presumably also linked by a disulphide bond. This arrangement of two intrachain disulphide bonds in the Fd fragment is in agreement with the work of Frangione & Milstein (1967), and the sequences around cysteine

Table 12. *Methods of isolation and composition of the chymotryptic peptides of Daw fragment 2a' tryptic peptides T2.5 and T2.p*

Mobility is given relative to lysine = +1.0, aspartic acid -1.0. The *S*-carboxyamidomethylcysteine content of the peptides is based on radioactivity. The tryptophan content of the peptides is based on the staining of the peptides on paper with Ehrlich's reagent.

Amino acid	Composition (mol of amino acid/mol of peptide)										
	Peptide T2.5	Peptide T2.5.C1	Peptide T2.5.C2	Peptide T2.5.C3	Peptide T2.p	Peptide T2.p.C1	Peptide T2.p.C3	Peptide T2.p.C4	Peptide T2.p.C5	Peptide T2.p.C1a	Peptide T2.p.C1b
Lys	0	0	0	0	1.1	0	0	1.2	1.1	0	0
Arg	1.1	0	1.0	1.0	0	0	0	0	0	0	0
Asp	1.8	1.9	0	0	1.3	1.4	0	0	0	1.0	0
Thr	2.4	2.6	0	0	1.9	0	0	1.7	0.7	0	0
Ser	0	0	0	0	5.1	0	2.4	3.0	2.2	0	0
Glu	0	0	0	0	2.0	1.0	0.9	0	0	0	1.1
Pro	1.3	1.2	0	0	0	0	0	0	0	0	0
Gly	2.2	2.3	0	0	2.9	2.2	1.2	0	0	0	2.2
Ala	2.2	1.1	1.1	1.0	1.3	0	0	1.2	1.0	0	0
Val	1.2	1.1	0	0	2.0	0	0	1.9	0	0	0
Ile	0	0	0	0	0.9	0.7	0	0	0	0	0.7
Leu	0	0	0	0	1.0	0.8	0	0	0	0	1.0
Tyr	1.8	1.8	0.9	0	1.6	0.9	0.7	0	0	1.0	0
Phe	0	0	0	0	0.9	0	0.8	0	0	0	0
Cmc	1	0	1	1	1	0	1	0	0	0	0
Trp	0	0	0	0	1	1	0	0	0	1	0
Total	15	12	4	3	24	8	7	9	5	3	5
Electrophoresis. Mobility at pH 3.5	+0.3	-0.33	+0.55	+0.65	—	-0.5	0	+0.14	+0.36	-0.3	0

Fig. 8. Amino acid sequence of some tryptic and chymotryptic peptides of Daw fragment 2a'. Sequence determination from the *N*-terminal end was by the 'dansyl'-Edman technique (→) or by digestion with leucine aminopeptidase (↔) and from the *C*-terminal end by digestion with carboxypeptidase A (←) or by hydrazinolysis (↔).

are very similar to those reported for other γ 1 heavy chains by these authors. The cysteine residue in fragment 5a must be present as a free thiol group

or perhaps linked to a cysteine residue, as found in a light chain (Milstein, Clegg & Jarvis, 1968).

Daw heavy chain has cysteine residues at positions

Table 13. *Chymotryptic peptides of Daw fragment 2a'*

S-Carboxyamidomethylcysteine residues are based on the radioactivity of the peptides. The tryptophan content of the peptides is based on staining of the peptides with Ehrlich's reagent.

Amino acid	Composition (mol of amino acid/mol of peptide)						
	Peptide C3.a	Peptide C4.f	Peptide C5.a	Peptide C5.d9	Peptide C5.e8	Peptide C5.g1	Peptide C6.d
Lys	0	1.1	0	0	0	0	0
His	0	0	0	0	0	0	0
Arg	0	0	0	0	0	0.8	0
Asp	2.0	0	1.0	0	0	0	1.2
Thr	2.6	1.0	0	0.9	0	0	0
Ser	0	3.1	0	0	0	2.3	0
Glu	0	0	1.2	0	1.1	1.0	0
Pro	1.0	2.3	0	0	0	0	0
Gly	2.1	1.4	2.0	0	1.9	1.2	0
Ala	1.0	1.1	0	0	0	1.0	0
Val	1.3	1.0	0	2.1	0	0	0
Ile	0	0	0.8	0	0.9	0	0
Leu	0	1.2	1.0	0	1.1	0	0
Tyr	1.0	0	1.0	0	0	0.7	0.9
Phe	0	0.8	0	0	0	0	0.9
Cmc	0	0	0	0	0	2	0
Trp	0	0	1	0	0	0	1
Total residues	11	13	8	3	5	9	4

Fig. 9. Sequence of the *N*-terminal part of Daw fragment 2a', showing tryptic and chymotryptic peptides.

22, 35, 97, 101, 146 and 201 (Fig. 10). The cysteine at position 221 in Daw heavy chain was shown by Steiner & Porter (1967) to be in disulphide linkage with the light chain. Presumably cysteine-22 is joined to cysteine-97, and cysteine-146 to cysteine-201, as shown for Cor heavy chain. The cysteine residues at positions 35 and 101 must also be linked by a disulphide bond, since it was shown by Piggot & Press (1967) that fragment 4 (residues 35-84) was linked to fragment 2a' (residues 85-225) in the Fab fragment.

Carbohydrate on Cor Fab fragment

Hexosamine was found in Cor fragment 5a, whereas the corresponding region of Daw, fragment

4, contained no hexosamine. The hexosamine content of whole IgG of Cor was 13.5mol/mol, of which 6mol/mol was on the Fc fragment and 7mol/mol on the Fab fragment. However, only 1.5mol of hexosamine/mol was found in fragment 5a, and so the other fragments of Cor Fd (5b, 6a and 2a') and the light chain were also analysed, and the hexosamine content was nil in all three fragments and in the light chain. We assume therefore that Fd fragment 5a had a similar amount of carbohydrate to that on the Fc fragment (Franklin, 1960, found 5mol of hexosamine/mol of normal Fc fragment), but that during digestion with cyanogen bromide in 70% formic acid and subsequent fractionation in 6*M*-urea on a column of Sephadex G-100 most of the carbohydrate was destroyed.

The neutral sugar content was not investigated. The carbohydrate was located on the chymotryptic peptide C3.c of fragment 5a (see section on the sequence of Cor fragment 5a). The residue involved in the linkage was determined by digesting totally reduced Fab fragment with chymotrypsin (1mg/ μ mol at 37°C for 4h) and with pronase (8mg/ μ mol at 37°C for 48h). The digest was fractionated on a column of Sephadex G-25 in 0.02M-ammonia, which separated the glycopeptide from the peptides, and refractionation on a column of Sephadex G-50 in 0.05M-ammonia to separate the glycopeptide from the enzymes. The only amino acid present in the glycopeptide was aspartic acid, and therefore the carbohydrate must be attached to the aspartic acid residue at position 62 in Cor Fd fragment. Presumably the linkage involves the β -carboxyl group of aspartic acid and *N*-acetylhexosamine.

DISCUSSION

The entire amino acid sequences of Daw and Cor Fd fragments are compared in Fig. 10. The numbering is based on Daw heavy chain, and to maximize the homology between the two a deletion of three

residues (88–90) and an insertion of four residues after position 104 have been placed in Cor heavy chain. There is only one difference between the two chains in the first 30 positions, namely a substitution of a lysine for an arginine residue at position 13. Then for residues 31–33 there are no residues in common between the two chains and thereafter there are blocks of identical residues, e.g. 38–44 and 64–69, interspersed with regions of differing residues. Altogether, 77% of the positions between 1 and 99 are occupied by the same residue in the two chains. The incidence of identical residues between positions 100 and 114 is very much less, only five or 30%, but from position 115 to the *C*-terminal residue the two chains have an identical sequence. The amino acid sequence of another γ 1 heavy chain, Eu, has been determined (Edelman *et al.* 1969) and this also has the same sequence as Daw and Cor from position 115; but a fourth γ 1 chain, He, for which a sequence of 21 residues was reported (Edelman *et al.* 1969), has an alanine residue instead of threonine at position 116. The variable region of the heavy chain must therefore extend at least to position 116. The variable regions of the light chains extend to positions 108 (κ chains) and 109 (λ chains), and there

	10	20	30
Daw	PCA-Val-Thr-Leu-Arg-Glu-Ser-Gly-Pro-Ala-Leu-Val-Arg-Pro-Thr-Gln-Thr-Leu-Thr-Leu-Thr-Cys-Thr-Phe-Ser-Gly-Phe-Ser-Leu-Ser-		
Cor	PCA-Val-Thr-Leu-Arg-Glu-Ser-Gly-Pro-Ala-Leu-Val-Lys-Pro-Thr-Gln-Thr-Leu-Thr-Leu-Thr-Cys-Thr-Phe-Ser-Gly-Phe-Ser-Leu-Ser-		
	40	50	60
Daw	Gly-Glu-Thr-Met-Cys-Val-Ala-Trp-Ile-Arg-Gln-Pro-Pro-Gly-Glu-Ala-Leu-Glu-Trp-Leu-Ala-Trp-Asp-Ile-Leu-Asn-Asp-Asp-Lys-Tyr-		
Cor	Ser-Thr-Gly-Met-Cys-Val-Gly-Trp-Ile-Arg-Gln-Pro-Pro-Gly-Lys-Gly-Leu-Glu-Trp-Leu-Ala-Arg-Ile-Asp-Trp-Asp-Asp-Asp-Lys-Tyr-		
	70	80	90
Daw	Tyr-Gly-Ala-Ser-Leu-Glu-Thr-Arg-Leu-Ala-Val-Ser-Lys-Asp-Thr-Ser-Lys-Asn-Gln-Val-Val-Leu-Ser-Met-Asn-Thr-Val-Gly-Pro-Gly-		
Cor	Tyr-Asx-Thr-Ser-Leu-Glu-Thr-Arg-Leu-Thr-Ile-Ser-Lys-Asp-Thr-Ser-Arg-Asn-Gln-Val-Val-Leu-Thr-Met-Asp-Pro-Val-Val-Thr-		
	100	110	115
Daw	Asp-Thr-Ala-Thr-Tyr-Tyr-Cys-Ala-Arg-Ser-Cys-Gly-Ser-Gln-Tyr-Phe-Asp-Tyr-Trp-Gly-Gln-Gly-Ile-Leu-Val-Thr-		
Cor	Asp-Thr-Ala-Thr-Tyr-Tyr-Cys-Ala-Arg-Ile-Thr-Val-Ile-Pro-Ala-Pro-Ala-Gly-Tyr-Met-Asp-Val-Trp-Gly-Arg-Gly-Thr-Pro-		
	120	130	140
Daw	Val-Ser-Ser-Ala-Ser-Thr-Lys-Gly-Pro-Ser-Val-Phe-Pro-Leu-Ala-Pro-Ser-Ser-Lys-Ser-Thr-Ser-Gly-Gly-Thr-Ala-Ala-Leu-Gly-Cys-		
Cor			
	150	160	170
Daw	Leu-Val-Lys-Asp-Tyr-Phe-Pro-Glu-Pro-Val-Thr-Val-Ser-Trp-Asn-Ser-Gly-Ala-Leu-Thr-Ser-Gly-Val-His-Thr-Phe-Pro-Ala-Val-Leu.		
Cor			
	180	190	200
Daw	Gln-Ser-Ser-Gly-Leu-Tyr-Ser-Leu-Ser-Ser-Val-Val-Thr-Val-Pro-Ser-Ser-Leu-Gly-Thr-Gln-Thr-Tyr-Ile-Cys-Asn-Val-Asn-His(Lys,		
Cor			
	210	220	225
Daw	Pro,Ser)Asn-Thr-Lys-Val-Asp-Lys-Lys-Val-Glu-Pro-Lys-Ser-Cys-Asp-Lys-Thr-His		
Cor			

Fig. 10. Comparative sequences of the Fd fragments of the two γ 1 heavy chains Daw and Cor. Identical residues are underlined for positions 1–114; for positions 115–225 the two fragments have an identical sequence.

Fig. 11. Comparison between the sequences of three $\gamma 1$ chains in the variable region, showing the highly-variable region (positions 100–104) and the probable persistence of subgroup sequences for positions 105–114. * Edelman *et al.* (1969).

since there is also good evidence that antibody specificity is related to primary structure it is reasonable to assume that the antigen binding site is within the variable regions of the Fd fragment and light chain. Within the variable region of light chains there are relatively constant regions and also positions that are apparently highly variable (Milstein, 1969). The comparative sequence study of Daw and Cor Fd fragments reveals a high incidence of variation for positions 100-104, in an analogous position to one of the light-chain hyper-variable regions, and perhaps also around positions 31-33, as noted above. These highly variable regions might be involved in antigen binding, or alternatively residues from many different positions in the primary sequence may contribute to form an antigen binding site in the three-dimensional structure. The solution of this problem will, however, require the application of some such technique as affinity labelling, in conjunction with sequence studies of antibodies.

The Fd fragment of Cor heavy chain has a carbohydrate moiety attached at position 62, which is unusual for a $\gamma 1$ heavy chain, although Abel, Spiegelberg & Grey (1968) also have evidence for the presence of carbohydrate on the Fd fragments of three gamma heavy chains. The attachment of carbohydrate on the variable region of light chains has also been reported in a few instances (Edmundson *et al.* 1968; Melchers, 1969; Hood, Grant & Sox, 1969). The sequence around the site of attachment of the carbohydrate on Cor Fd fragment is Tyr-Asx-Thr-Ser, which is very similar to the sequence around the carbohydrate on the Fc fragment of human and rabbit heavy chains, namely Tyr-Asn-Ser-Thr and Phe-Asx-Ser-Thr (Edelman *et al.* 1969; Hill, Delaney, Fellows, & Lebovitz, 1966) and on the mouse light chain, Gln-Asx-Ile-Ser (Melchers, 1969). Comparison of glycopeptides from heavy chains of several species is consistent with the sequence -Asx(CHO)-X-Ser (Thr)- as the site of attachment of carbohydrate, where X can be one of several amino acids (Howell, Hood & Sanders, 1967), and it was suggested by Neuberger & Marshall (1968) that this sequence might be a recognition site for an enzyme involved in the attachment of hexosamine to proteins. However, bovine ribonuclease B contains a carbohydrate moiety attached to an aspartic residue, whereas in ribonuclease A the same sequence occurs, namely Asn-Leu-Thr-Lys, without any carbohydrate attached (Plummer & Hirs, 1964). Presumably, therefore, sequence is not the only requirement for carbohydrate attachment to protein chains.

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