Structure of Heavy Chain from Strain 13 Guinea Pig Immunoglobulin-G(2). III. Amino Acid Sequence of the Region around the Half-Cystine Joining Heavy and Light Chains

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ABSTRACT: The amino acid sequence of a 65-residue segment from the second quarter of the heavy chain of guinea pig IgG(2) has been determined. This segment includes a 44-residue cyanogen bromide fragment (C-1-b) and a 28-residue tryptic peptide (T36–37).

The tryptic peptide contained two contiguous methionine residues and was shown to extend the amino acid sequence of γ_2 chain from C-1-b toward the N terminus by 21 residues. T36–37 had a constant sequence and was placed at a position close to the switch point, that position in myeloma proteins N terminal to which heavy-chain subgroups have variable sequences. C-1-b was shown to be joined by an intrachain disulfide bond to another cyanogen bromide fragment, C-1-c.

Comparison of the available sequences for all or part of the heavy chains from human IgG(1)[1] myeloma proteins has led to the conclusions that the N-terminal ~120 residues of this subclass may have a "variable" sequence and that these molecules may be grouped on the basis of relatedness of this section to one of several reference proteins (Press and Hogg, 1969; Cunningham *et al.*, 1969). Since each recognized subgroup of variable region appears to occur in the serum of every human examined, the assumption has been made that these subgroups are polymorphic forms controlled by separate genes (Milstein and Feinstein, 1969; Grant and Hood, 1970). In order to demarcate further the section(s) of heavy chain from normal guinea pig IgG(2) involved in determining the specificity of antibodies, the possible occurrence of subgroups of the N-terminal 120 residues must be assessed, and peptides coming from this section must be recognized. To this end, the established sequence of C-1-c (Turner and Cebra, 1971), the 65-residue fragment at the C-terminus of Fd, was extended toward the N-terminal end of heavy chain by another 65 residues to a point judged to correspond closely to the junction with the variable region of myeloma heavy chains. These present sequence data should permit the placement of CNBr fragments and tryptic peptides within the section corresponding to the variable region of myeloma heavy chains and allow the recognition of alternate sequences reflecting subgroups in normal IgG(2).

Materials and Methods

Preparation of C-1-b. The fragment C-1-b was prepared from a CNBr digest of mildly reduced, radioalkylated γ_2 chain as outlined in detail previously (Birshtein *et al.*, 1971).

Tryptic Digest of γ_2 Chain. Heavy chain was prepared from IgG(2) by mild reduction and radioalkylation of easily reduced half-cystine residues (Birshtein *et al.*, 1971). The remaining disulfide bonds were then reduced with dithiothreitol and alkylated with cold iodoacetic acid. Immediately after alkylation, the heavy chain (165 mg) was dialyzed against distilled water to remove excess reagents and guanidine. The pH of this protein solution (~10 mg/ml) was adjusted to 8.1 with 0.1 N NaOH, and the digestion was carried out at 37° in an autotitrator (Radiometer, Copenhagen) with the addition of four 1.5-mg aliquots of trypsin (from a stock solution of 6 mg/ml in 0.005 M $MgSO_4$). Digestion was continued until further addition of trypsin did not result in uptake of base.

The digest was applied to a column of Sephadex G-50 (3.4 × 180 cm) equilibrated in 0.05 M NH_4HCO_3 (Figure 1). Pool I, containing the largest radiolabeled peptides, was recycled through the same column before its further purification.

Ion-Exchange Chromatography of Enzymic Digests on Columns of Dowex 1-X2. Dowex 1 was graded according to the procedure of K. Titani (personal communication). Dowex 1-X2 acetate (200–400 mesh; AG 1-X2, Bio-Rad, Richmond, Calif.) (450 g) was suspended in 2 l. of water in a tightly covered 2-l. graduated cylinder. After the cylinder was inverted five times, the resin was allowed to settle for 10 min. The supernatant was drawn off into a 2-l. graduated cylinder, its volume brought to 2 l. with water, and the procedure of inversion–settling–drawing off was repeated on each resulting supernatant for a total of five times. The resin that remained in the final supernatant was again brought to a volume of 2 l. in water and permitted to settle for 30 min. The sedimented material was used for ion-exchange chromatography of enzymic digests.

Freeze-dried pool I from the tryptic digest of γ_2 chain was dissolved in 3% pyridine and applied to a column of Dowex 1-X2 (0.6 × 135 cm) equilibrated in the same solvent. The column was developed at a rate of 1.0 ml/min, initially with 3% pyridine. After peptides not retarded by the resin were eluted, the column was developed with a continuous gradient of 3% pyridine, pyridine acetate (0.1 N in pyridine, 0.05 N in acetic acid), pyridine acetate (0.9 N in pyridine, 0.5 N in

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[1] The nomenclature is in accord with that suggested in *Bull. W. H. O.* (1964), *30*, 447, and with that proposed by the Conference on Nomenclature for Animal Immunoglobulins, Prague, June 1969.

TABLE 1: T36–37 and Peptides Derived from It by Chymotryptic and Thermolytic Digestion.

	T36–37	Ch3	Ch4	Ch5	Ch6	Th2	Th3	Th4	Th5	Th6	Th7	Th17	Th18[d]
Lys	1.1			0.62[a]	0.92						1.1		
CMCys	1.8		0.80	0.33	0.47								
Asp	1.1		1.0						1.0			1.0	
Thr	4.0	2.0	0.94	0.92		1.8			1.7			1.2	1.2
Ser	3.9	1.1	2.2			1.3		1.2	2.1			1.7	
Pro	2.0	2.2				0.92	1.0						
Gly	2.1		1.0	1.1	1.2				1.1	1.2		1.1	
Ala	2.9	1.1	1.4			0.89		2.1					
Val	2.7	1.0	1.0	1.0	0.92		1.2		1.0		1.0	0.86	
Met	1.7		1.1									0.80	0.84
Leu	3.0	1.0		1.9	0.93			0.86		1.0	0.84		
Phe	0.93	1.0					0.89						
$CySO_3$								0.81		0.92			
Met-sulfone									1.6				
Mobility[b]													
pH 3.6		+0.06		+0.17	+0.24	+0.15	+0.27	−0.86	−0.18	−1.18	+0.81	−0.19	+0.32
pH 6.5	−0.22											−0.35	

Compositions are reported as moles of amino acid per mole of peptide. Mobility given relative to lysine = +1.0, aspartic acid = −1.0, neutral amino acid = 0. [a] The lysine residue was determined after peptide Ch5 had undergone three Edman degradation steps. [d] Peptides Th3 and Th18 were not resolved by electrophoresis at pH 3.6. They were separated by preparative descending chromatography in a solvent of 1-butanol–acetic acid–water (4:1:1, v/v). Peptide Th18 has R_F 0.58 in this system.

acetic acid), and pyridine acetate (2.0 N in pyridine, 2.0 N in acetic acid) (K. Titani, personal communication). This gradient of increasing pyridinium acetate concentration and decreasing pH was prepared in an Autograd (Technicon, Ardsley, N. Y.). Two of eight chambers of the Autograd were filled with a total of 300 ml of each solution, beginning with 3% pyridine in the two chambers nearest the outflow tube. Fractions were collected every 7.5 min from the portion of the effluent not used for analysis. A portion (0.16 ml/min) of the effluent was continuously taken and automatically analyzed by reaction with ninhydrin after alkaline hydrolysis in a Technicon AutoAnalyzer (Catravas, 1964).

Radioactivity of the effluent fractions was measured as described previously (Birshtein *et al.*, 1970).

Ion-Exchange Chromatography in Columns of Dowex 50-X8. The enzyme digest was applied to a column (0.9 × 65 cm) of Dowex 50-X8 (AA-15, Beckman), equilibrated in pyridine acetate (0.05 M in pyridine, pH 2.4). The column was developed at 1.4 ml/min, initially with pyridine acetate (0.05 M in pyridine, pH 2.4). After peptides not retarded by the resin had been released, the column was developed with a linear gradient using 750 ml each of pyridine acetate (0.05 M in pyridine, pH 2.4) and pyridine acetate (1.0 M in pyridine, pH 4.0). Fractions were collected for 7.5 min each from the portions of the effluent not used for analysis. A portion of the effluent (0.16 ml/min) was automatically analyzed by reaction with ninhydrin after alkaline hydrolysis in a Technicon AutoAnalyzer.

Other Methods. Tryptophan was detected in peptides after their electrophoresis on paper by the Ehrlich stain (Easley, 1965).

Tryptic, chymotryptic, and thermolytic digests of C-1-b and T36–37 were made as described in the preceding paper (Turner and Cebra, 1971).

T36–37 was oxidized with performic acid as described by Moore (1963).

High-voltage electrophoresis of peptides at pH 3.6 and 6.5 and amino acid sequence analyses of component peptides were carried out as previously described (Turner and Cebra, 1971).

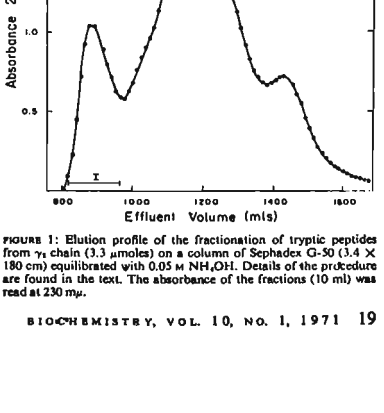

FIGURE 1: Elution profile of the fractionation of tryptic peptides from γ_1 chain (3.3 μmoles) on a column of Sephadex G-50 (3.4 × 180 cm) equilibrated with 0.05 M NH_4OH. Details of the procedure are found in the text. The absorbance of the fractions (10 ml) was read at 230 mμ.

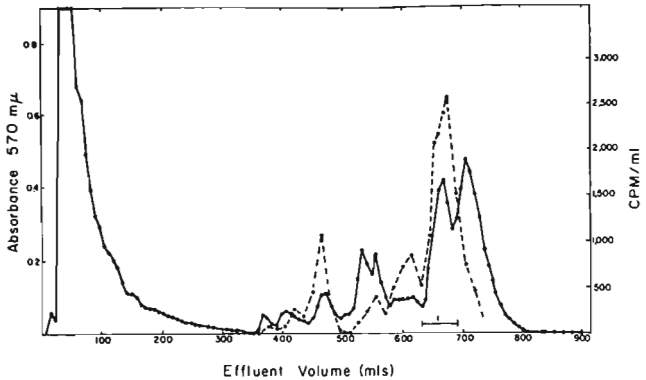

FIGURE 2: Separation of the tryptic peptides of γ_1 contained in pool I (Figure 1) on a column of Dowex 1-X2. Details of the procedures are found in the text. The column was developed at 1 ml/min with a continuous gradient of pyridinium acetate buffers. A portion of the effluent (0.14 ml/min) was automatically analyzed by the ninhydrin reaction after alkaline hydrolysis. The absorbance of the reaction mixture was monitored at 570 mμ as shown by the solid line. The dashed line indicates the radioactivity as measured on 0.1-ml aliquots of every other tube. Fractions were collected for 7.5 min each.

Pyrrolidonecarboxylic acid hydrolase was a gift of Dr. James Prahl. Peptide Ch12 (0.3 μmoles) was dissolved in 2.0 ml of 0.05 M sodium phosphate (pH 7.3), 0.01 M in 2-mercaptoethanol and 0.001 M in EDTA. The enzyme was added to the sample, and the mixture was incubated at 37° for 3.5 hr. After incubation, the sample was adjusted to pH 3.0 with acetic acid and was applied to a column of Dowex 50-X2 (0.8 × 6 cm) (small bead form) equilibrated in water. The column was washed first with water and then with 1 M NH_4OH. Peptide Ch12, minus its N-terminal pyrrolidonecarboxylic acid, was eluted with 1 M NH_4OH.

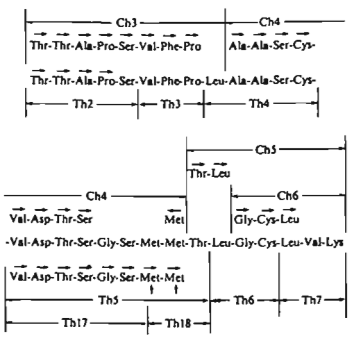

FIGURE 3: A schematic representation of the procedures and resulting data used to determine the sequence of T36-37. Exact compositions of the peptides are given in Table I. →, removal of a residue by the Edman degradation procedure; ←, identification of a residue by treatment with carboxypeptidase A; ↑, confirmation of a residue by dansylation. The residues in this fragment and its component peptides were ordered using the subtractive Edman procedure.

The nomenclature of peptides in this paper is that described by Turner and Cebra (1970).

Results

Preparation of T36–37. Resolution by gel filtration of the tryptic digest of totally reduced and alkylated γ_1 chain is depicted in Figure 1. Pool I, after being recycled through the same column, was subjected to ion-exchange chromatography on Dowex 1-X2. Pool 1 (Figure 2) contains a major radiolabeled peptide. After preparative paper electrophoresis at pH 6.5, the eluted peptide, T36–37, gave the amino acid composition and electrophoretic mobility as shown in Table I.

Amino Acid Sequence of T36–37. The N-terminal sequence was determined directly for four residues as schematized in Figure 3. Intact T36–37 was then subjected to chymotryptic digestion. The digest was applied to a column of Sephadex G-25, fine (2 × 240 cm), equilibrated in 0.05 M NH_4OH. Figure 4 shows the two major pools resulting from the filtration. Pool A contained a single peptide, Ch4, whose analysis can be found in Table I. The mixture of peptides found in pool B was resolved by paper electrophoresis at pH 3.6. Peptides Ch3, Ch5, and Ch6 were purified in this way, and their mobilities and amino acid analyses can be found in Table I. These peptides were sequenced by the subtractive Edman procedure as shown in Figure 3. Peptides Ch5 and

TABLE II: C-1-b, Component Tryptic Peptides, and a Thermolytic Peptide from T35.

	C-1-b	T35	Th8	T36
Lys	2.6	1.0	0.91	0.91
His	0.85			
Arg	0.30			
CMCys	1.1			0.66
Asp	1.2			
Thr	4.2	0.95		0.98
Ser	4.3			
Glu	2.2	1.0		
Pro	3.1	2.0		
Gly	4.7	1.1		1.1
Ala	2.2			
Val	4.6	1.8	1.1	1.3
Leu	5.1			1.5
Tyr	1.7	1.0		
Phe	1.9	0.97		
Hse	1.0			
Trp	+			
Mobilities pH 3.6		+0.26	+0.87	+0.21

Compositions and mobilities are expressed as indicated in Table I.

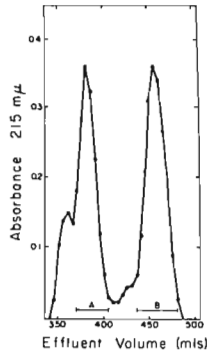

FIGURE 4: Elution profile of the separation of the peptides resulting from a chymotryptic digest of T36-37 (0.7 μmole) on a column of Sephadex G-25, fine (2.0 × 240 cm). Details of the procedure are found in the text. Fraction size was 5 ml.

Ch6 both contained lysine and consequently were derived from the C-terminal end of the parent tryptic peptide. Since peptide Ch3 had an N-terminal sequence of Thr-Thr-Ala-Pro identical with that of the parent molecule, it was the N-terminal chymotryptic peptide. Peptide Ch4 contained all the radiolabel of the parent tryptic peptide. Its C-terminal amino acid was methionine as determined by carboxypeptidase A treatment. The sequence of its N-terminal eight residues was determined to be that shown in Figure 3.

The analysis of T36–37 (Table I) indicated the presence of two methionyl residues. To confirm this as well as to complete the sequence, T36–37 was oxidized with performic acid to convert methionine into methionine sulfone and thus make this residue unsusceptible to the subsequent thermolytic cleavage (Ambler and Meadway, 1968). The thermolytic digest of oxidized T36–37 was resolved at pH 3.6 with high-voltage paper electrophoresis. The analyses and mobilities of component peptides Th2–7 are shown in Table I. Figure 3 shows that peptide Th4 established the overlap between peptides Ch3 and Ch4, and that peptide Th6 confirmed the overlap between peptides Ch5 and Ch6. Peptide Th5 contained two methionine sulfone residues and was sequenced directly as shown in Figure 3. The C-terminal Thr of peptide Th5 is the N-terminal residue of peptide Ch5. Thus the complete alignment of the chymotryptic peptides of T36–37 is N-Ch3-Ch4-Ch5-C.

Further confirmation of the presence of two methionine residues and the determination of Asx as Asp came from a thermolytic digest of intact, unoxidized T36–37. The digest was resolved at pH 3.6 with high-voltage paper electrophoresis. The analyses and mobilities of peptides Th17 and Th18 are shown in Table I. The origin of these peptides is schematized in Figure 3. High-voltage electrophoresis at pH 6.5 of peptide Th17 indicated its mobility to be −0.35 and thus established Asx as Asp. Consequently, the complete equence of T36–37 is that shown in Figure 3.

Position of Cyanogen Bromide Fragment C-1-b. The amino acid composition of C-1-b is given in Table II. This fragment was digested with trypsin and the resulting peptides were fractionated by gel filtration on a column of Sephadex G-25, fine (2.0 × 240 cm), equilibrated with 0.05 M NH_4OH. Two fractions were obtained, which eluted at 1.0 and 1.2 void volumes. The first fraction eluted proved to be poorly soluble in aqueous buffers and difficult to purify. Its content of homoserine suggested that it contained the C-terminal portion of C-1-b. This section, T34, containing 27 residues, was sequenced using other enzymic digests. The fraction which eluted at 1.2 column volumes contained the two tryptic peptides T35 and T36. They were resolved by preparative electrophoresis at pH 3.6 and their compositions and relative mobilities are given in Table II. The composition of T36 was the same as that of Ch5, a peptide obtained from a chymotryptic digest of peptide T36–37 (*vide supra*). Since neither the sequence nor composition of the N-terminal ends of CNBr fragments C-1-c, C-3, C-4, and C-5 could accommodate peptide Ch5, it was probable that the sequence of T36–37 extended into that of C-1-b. It will be shown that peptide T36 is the N-terminal tryptic peptide of C-1-b. Thus this CNBr fragment extends the sequence of γ_1 chain C-terminal to T36–37.

Sequence of C-1-b. The sequence of T36 was taken as that of Ch5. The first seven residues of peptide T35 were determined directly as indicated in Figure 5. The entire sequence of T35 was obtained by isolating the peptide Th8 with the sequence Val-Lys from a thermolysin digest of T35. The peptide Th8 was prepared by paper electrophoresis at pH 3.6 and was the most basic component in the digest (Table II).

The Glx residue at position N-5 of T35 was identified as Glu from the behavior of the component peptide Th11 (Figure 5) upon paper electrophoresis at pH 6.5. The relative mobility of intact Th11 was −0.10 and after one and two

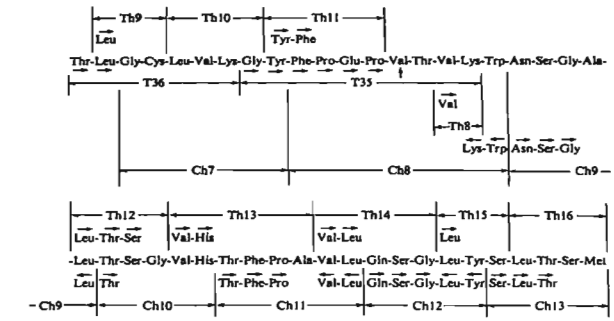

FIGURE 5: A schematic representation of the procedures and resulting data used to determine the sequence of C-1-b. Exact compositions of peptides are given in Tables II and III. The symbols are those used in Figure 3, except that ← also refers to identification of a residue by treatment with carboxypeptidase B. The subtractive Edman procedure was used throughout. Occasionally, as shown, dansylation was used for confirmation.

degradative steps this became −0.29 and −0.45, respectively.

To sequence the remaining 27 residues of C-1-b and to align the tryptic peptides T34, T35, and T36, separate 2-μmole amounts of C-1-b were digested with chymotrypsin or thermolysin. Both digests were fractionated on Dowex 50-X8. The resolution of the thermolytic digest is depicted in Figure 6, and that of the chymotryptic digest in Figure 7. Final purification of many of the peptides was achieved by preparative paper electrophoresis. Table III gives the compositions, Dowex pool of origin as marked in Figures 6 and 7, and relative mobilities of the collection of peptides from both digests that were used to complete the sequence of C-1-b.

Figure 5 illustrates how, in accord with the known specificities of thermolysin and chymotrypsin, the peptides from one digest overlapped those from the other and thus permitted an alignment of the peptides from each digest. The sequence for the section comprising T34 was obtained using the constituent thermolysin and chymotryptic peptides as schematized in Figure 5.

Peptide Ch12 was isolated as an acidic, ninhydrin-negative, tyrosine-positive peptide with the composition (Glx,Ser,Gly, Leu,Tyr). Hydrolysis with carboxypeptidase A for various times led to the C-terminal sequence Leu-Tyr. The whole peptide was then subjected to the action of pyrrolidonecarboxylic acid hydrolase and the residual peptide was purified on Dowex 50-X2. It had the composition (Ser,Gly, Leu,Tyr). This peptide, with a free α-amino group, was then used to complete the sequence of Ch12. The Glx was identified as glutamine from the neutral behavior of Th14 upon electrophoresis at pH 6.5 (Table III). The Asx in peptide Ch9 was likewise designated as asparagine based on its migration as a neutral peptide at pH 6.5.

The C-terminal sequence of Ch8 was established as Lys-Trp by the consecutive action of carboxypeptidase A and carboxypeptidase B which yielded stoichiometric recoveries of Trp and Lys in a molar ratio of 1.2:1.0. Peptide Ch8 was used to align T35 with the T34 section. Although no formal alignment of Ch8 and Ch9 has been established, the rest of the peptides of C-1-b have been rigorously aligned. Since the component peptides of C-1-b account for the overall composition of this fragment, the order and sequence across the Trp-Asn are likely to be as given in Figure 5. Peptides T36 and T35 were formally aligned as N-T36-T35-C using peptides Th10 and Ch7.

Position of the Intrachain Bond Formed with the Half-Cystine Present in C-1-b. The *S*-carboxymethylcysteine residue in purified C-1-b contains no radioactivity, even though the starting γ_2 chain was obtained by mild reduction and subsequent carboxymethylation of IgG(2) with iodoacetic-^{14}C acid so that about four half-cystine residues in the chain were labeled (Birshtein *et al.*, 1971). Thus it is likely that the half-cystine in C-1-b participates in forming an intrachain disulfide bond. In the fractionation step of the CNBr digest immediately prior to the final purification step for C-1-b, this fragment occurs together with C-1-c even though the two fragments have lengths of 44 and 65 residues, respectively (see Figure 3, Birshtein *et al.*, 1971). This fractionation step consists of gel filtration on a column of Sephadex G-75 in 0.05 M NH_4HCO_3. The fragment C-1-b is only separable from C-1-c by gel filtration on Sephadex G-75 or G-50 in 0.05 M NH_4HCO_3 after complete reduction and carboxymethylation of disulfide bonds (see Figure 4, Birshtein *et al.*, 1971). Thus it is likely that C-1-b and C-1-c are joined by an inter-fragment disulfide bond. Table IV lists the composition of the fragments C-1-b and C-1-c, known from their sequences, and the sum of these compositions and compares this with the amino acid compositions obtained for the supposed C-1-b–C-1-c fragment. The composition of this supposed C-1-b–C-1-c fragment compares well with the sum of its components. Thus the complex of C-1-b–C-1-c contains a disulfide bond which was an intrachain disulfide bond before CNBr digestion. The only half-cystine present in C-1-c which is not radiolabeled on mild reduction and

TABLE III: Chymotryptic and Thermolytic Peptides from C-1-b.

	Ch7	Ch8	Ch9	Ch10	Ch11	Ch12	Ch13	Th9	Th10	Th11	Th12	Th13	Th14	Th15	Th16
Lys	1.1	1.1							0.84						
His				0.90								0.92			
CMCys	0.60							0.72							
Asp			1.1												
Thr		1.0		1.0	1.0		1.1				0.89	1.0			1.0
Ser			1.0	1.0		0.95	1.8				1.1		0.90	1.0	1.0
Glu		1.1				1.0				1.0			1.0		
Pro		1.7			1.0					2.0		1.1	0.45		
Gly	2.1		1.0	1.2		1.0		1.2	1.0		1.1		1.1		
Ala			0.90		1.2							1.0			
Val	1.0	2.1		1.0	1.0				1.1			0.94	1.0		
Leu	1.0		0.90		1.0	1.2	1.0	0.82	1.1		1.0		1.2	1.1	0.93
Tyr	0.90					0.78				0.80				0.89	
Phe		0.90			0.90					1.2		0.92			
Hse							0.75								0.60
Trp		+													
Mobility															
pH 3.6	+0.16	+0.32	+0.28	+0.55	+0.16	−0.72	+0.54	−0.70							
pH 6.5			0							−0.10			0		
Pool no.[b]	9	8	4	9	2	1	4	9	19	5	7	18	4	13	6

Compositions and mobilities are expressed as indicated in Table I. [b] These numbers refer to the pools of effluent containing each peptide made after Dowex 50 chromatography. See Figure 7 for separation of chymotryptic digest and Figure 6 for separation of thermolytic digest.

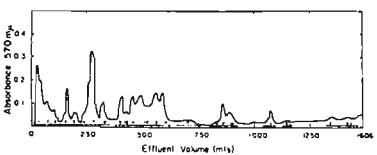

FIGURE 6: Fractionation of the thermolytic digest of C-1-b (2 μmoles) on a column of Dowex 50-X8. Details of the procedure are found in the text. The column was developed at 1.4 ml/min with a linear gradient of pyridine acetate buffers. A portion of the effluent (0.16 ml/min) was automatically analyzed by the ninhydrin reaction after alkaline hydrolysis. The absorbance of the reaction mixture was monitored at 570 mμ. Fractions were collected for 7.5 min each.

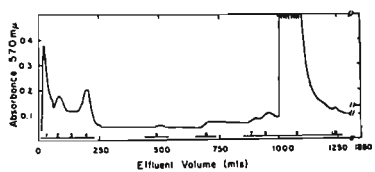

FIGURE 7: Fractionation of the chymotryptic digest of C-1-b (2 μmoles) on a column of Dowex 50-X8. Details of the procedure are found in the text and in the legend to Figure 6.

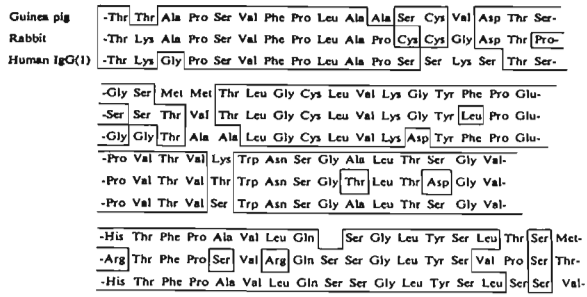

FIGURE 8: The region in guinea pig γ_1 chain around the half-cystine (N-13) which joins it to light chain as compared to homologous regions in rabbit IgG and human IgG(1). When any two or three residues are identical at a given position, they are included in the closed rectangles.

TABLE IV: Composition of C-1-b–C-1-c Complex, Disulfide Peptide, and Components Derived from Them after Reduction and Carboxymethylation.

	C-1-b[a]	C-1-c[a]	Sum[a]	C-1-b–C-1-c[b]	T32[a]	T35[a]	Sum[a]	T32–T35[b]
Lys	2	6	8	10.0	1	1	2	2.0
His	1	1	2	1.6	1		1	1.1
Arg	0	1	1	1.6				
CMCys	1	4	5	2.2	1	1	2	
Asp	1	5	6	6.5	1		1	1.5
Thr	5	7	12	11.4	2	1	3	2.4
Ser	5	5	10	8.4	2		2	2.2
Glu	2	4	6	7.4				
Pro	3	14	17	16.0	1		1	1.0
Gly	5	2	7	6.8		1	1	1.3
Ala	2	3	5	5.3	3		3	3.4
Ile	0	2	2	2.4				
Leu	6	2	8	8.0		2	2	1.3
Val	5	6	11	11.0	1	1	2	2.4
Tyr	2	0	2	2.5				
Phe	2	2	4	4.5				
Hse	1	1	2	2.3				
Cys				+				

These compositions are taken from the sequences established for C-1-b and C-1-c (Turner and Cebra, 1971) and their component tryptic peptides. [b] Actual compositions are expressed as moles of amino acid per mole of peptide.

radioalkylation is in tryptic peptide T32. To confirm the disulfide bond between T32 of C-1-c and T35 of C-1-b, the C-1-b–C-1-c complex was subjected to tryptic digestion and the disulfide-containing peptide was isolated. The digest was first fractionated by gel filtration on a column of Sephadex G-50 from which the disulfide-containing peptide was eluted at 1.8 column volumes. Peptides T28–29 and T31 were eluted from this same column at 1.5 and 2.1 column volumes, respectively. The supposed T32–T35 disulfide peptide was further purified by paper electrophoresis at pH 3.6 (relative mobility +0.49). Its composition is given in Table IV and compared to the expected sum of the compositions of T32 and T35 obtained from their sequences. The agreement of the compositions is close and confirms the placement of the disulfide bond between T32 and T35.

Discussion

The sequence of the 65 residues contained in T36–37 and C-1-b extends the constant region of guinea pig γ_1 chains and of its Fd section—N terminal to the 65 residues of C-1-c, whose sequence has been reported (Turner and Cebra, 1971).

The isolation of C-1-b joined to C-1-c by a disulfide linkage and the isolation of the cystine-containing tryptic peptide which contained this bond have demonstrated the presence of an intrachain disulfide bridge linking residues ~N-146 to N-201 (protein Daw numbering).

By comparison to the amino acid sequences of the heavy chains of human myeloma proteins Daw (Press and Hogg, 1969) and Eu (Edelman *et al.*, 1969), this section containing C-1-b and T36–37 spans the region from residue N-187 (Daw) or N-185 (Eu) to residue N-122 (Daw) or N-120 (Eu). The N terminus of T36–37 is thus close to the "switch point"—that position N terminal to which myeloma heavy chains of the same subgroups have different sequences. The position of the switch point has not been precisely assigned; Press and Hogg (1969) place it at N-114–115 (Daw) and Cunningham *et al.* (1970) at N-118–119 (Eu). Nevertheless, the isolation of peptide T36–37, which extends from ~N-120 to N-149 and contains two adjacent methionines, should define that CNBr fragment from guinea pig γ_2 chain which extends into the region corresponding to that called "variable" in human myeloma proteins. Since T36–37 has a constant sequence and contains a half-cystine readily labeled upon mild reduction and radioalkylation, it promises to be helpful as a marker for the identification and placement of fragments accounting for the N-terminal ~120 residues of γ_2 chain. This is the section likely to be involved in antibody specificity and is where a radioactive affinity label is localized (A. Ray, unpublished data).

Positions N-12 and N-13 in the sequence given in Figure 8 are of especial interest because of the presence or absence of half-cystines. In guinea pig γ_2 chain, the half-cystine at position N-13—corresponding to N-134 (Daw)—participates in the disulfide bridge linking it to light chain (Oliveira and Lamm, 1971). In the rabbit γ chain, the half-cystines at positions corresponding to N-12 and N-13 of Figure 8 link heavy to light chain as well as form a labile intra-heavy chain disulfide bridge (O'Donnell *et al.*, 1970). Human IgG(1) lacks these half-cystines entirely, and its heavy–light disulfide bridge is formed at a position further toward the C terminus, near the middle of γ_1 chain (Steiner and Porter, 1967). Other subclasses of human myeloma proteins appear to have interchain disulfide bonds linking heavy to light chains at positions corresponding to those of the rabbit and guinea pig protein (Frangione *et al.*, 1969).

Finally, Figure 8 presents a comparison of the sequence from guinea pig γ_2 chain reported here with corresponding sequences from rabbit IgG (Cebra *et al.*, 1968; Fruchter *et al.*, 1970) and human IgG(1) (Edelman *et al.*, 1969). The section depicted is the one initially used to point out the homologies occurring among Fd, Fc, and light chains (Cebra, 1967). The faithful conservation of certain sections of this sequence among species is quite striking.

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