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Primary Structure of the C_H2 Homology Region from Guinea Pig IgG2 Antibodies†

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ABSTRACT: The amino acid sequence of a 93-residue cyanogen bromide fragment, C-3, from the Fc region of the heavy chain of strain 13 guinea pig IgG2 has been determined. This fragment spans the region ~N-249 to ~N-341 of the γ_2 chain. It contains one disulfide bond which spans a 61-residue section from ~N-257 to ~N-317 and includes the only known attachment point for oligosaccharides in the γ_2 chain. The fragment appears to have a single sequence which is about 69% homologous with corresponding regions of either rabbit IgG or human IgG1. As this fragment C-3 of the heavy chain corresponds to a large portion of the C_H2 homology region, it may be possible to dimerize it to form a C_H2/C_H2 domain. This domain may be useful in attempts to relate to antibody structure various biologic functions of guinea pig IgG2, notably the initiation of complement activation by the conventional pathway.

A major aim of our determining the primary structure of the heavy chain from normal strain 13 guinea pig IgG2 has been to establish the positions of single residues or groups of residues which had alternative amino acids or amino acid sequences. At this point, all our analyses are consistent with there being a single sequence for the ~327 residues comprising the carboxyl-terminal three-quarters of the γ_2 chain, and we have demonstrated variability at certain residue positions within the amino-terminal quarter, or V_H region, of the chain.

To accomplish a primary structural analysis of the entire γ_2 chain we have isolated eight cyanogen bromide fragments from *normal* heavy γ_2 chain (Birshtein *et al.*, 1971a) which have been formally aligned (Benjamin *et al.*, 1972) and which account for the entire γ_2 chain. The three amino-

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terminal fragments, C-1-n, C-1-a_1, and C-1-a_2, have been found to contain markedly variable segments (Cebra *et al.*, 1971; Birshtein and Cebra, 1971; Koo and Cebra, 1974; P. Koo, A. Ray, T. Trischmann and J. Cebra, unpublished data). In addition, their importance in determining the antigen-binding site has been implied by the specific localization of affinity label exclusively to these fragments (Ray and Cebra, 1972; Koo and Cebra, 1974).

The complementary part of our findings has led to the demonstration that fragments derived from the carboxyl-terminal three-quarters of the γ_2 chain appear to have a single sequence. In addition to the elucidation of the primary structures of fragments C-1-b (Birshtein *et al.*, 1971b) and C-1-c (Turner and Cebra, 1971), a single sequence was established for a tryptic peptide spanning ~N-120 to ~N-147 (Birshtein *et al.*, 1971b), which extended the established constant region sequence from ~N-120 to ~N-248. The amino acid sequence of C-3, reported in this paper, extends the established sequence from ~N-249 to ~N-341. The primary structure of the region from ~N-342 to ~N-426 (fragment C-4) has just been determined (Trischmann and Cebra, 1974). Finally, the amino acid sequence of the carboxyl-terminal octadecapeptide, C-5, which extends from ~N-427 to ~N-444, has already been reported (Turner and Cebra, 1971).

The availability of amino acid sequence data from the constant homology regions of γ_2 chain will enable comparisons to be made with the primary structure of γ_1 chain from guinea pig IgG1. Such comparisons may lead to the correlation of structure with those distinct secondary biological activities to be first associated with different immunoglobulin isotypes, the guinea pig IgG1 and IgG2 molecules. These activities, normally expressed after antibody–antigen interaction, include the initiation of complement activation by the conventional pathway by IgG2 (Bloch *et al.*, 1963) and the participation of IgG1 in anaphylactic reactions (Ovary *et al.*, 1963). We have isolated and tentatively aligned all of the component fragments of the IgG1 heavy chain (D. Tracey, S. Liu, and J. Cebra, unpublished data) and are presently engaged in the primary structural analysis of the cyanogen bromide fragments from its constant region.

Materials and Methods

Preparation of C-3. IgG2 was isolated by passage of serum through a column of DEAE-cellulose equilibrated in urea–phosphate buffer. Heavy and light chains, after mild reduction and alkylation, were separated on a column of Sephadex G-75 equilibrated in 1 M propionic acid. A cyanogen bromide digest of heavy chains was fractionated on a column of Sephadex G-100 equilibrated in 8 M urea–0.1 M formic acid. Components of a pool from the G-100 column containing C-3 were passed through a column of Sephadex G-75 equilibrated in 0.05 M NH_4HCO_3. C-3 was isolated cleanly in a pool of effluent from the G-75 column. The complete details of these procedures have been previously published (Birshtein *et al.*, 1971a). The fragment retains an intact disulfide bond when isolated as described.

Enzymic Digestion of C-3. Fragment C-3 (8 μmol) was first reduced with 0.05 M dithiothreitol and the half-cystines which participated in the cleaved disulfide bond were marked by carboxymethylation with 0.11 M [^{3}H]iodoacetic acid (100 μCi, New England Nuclear Corp.). Both steps of this reaction were carried out in 7 M guanidine·HCl 0.1 M in Tris-acetate (pH 8.0). Salts were removed by gel filtration through a column of Sephadex G-25 coarse equilibrated in 0.05 M NH_4HCO_3. Radioactivity was measured in a liquid scintillation counter using Kinard's (1957) fluid. The solution containing C-3 was lyophilized and redissolved in a small volume of 0.005 M NH_4HCO_3 (pH 8.0).

Reduced and radioalkylated C-3 (3.4 μmol) was digested with trypsin (Worthington Biochemical Corp.) which had been treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)[1] (Cyclo Chemical Corp.) before use (Kostka and Carpenter, 1964). The digestion was carried out at 37° for 6 hr by the addition at 0 and 3 hr of 50 μg of TPCK-trypsin, and the pH of the solution was adjusted to 8.0 15 min after each addition.

Reduced and radiocarboxymethylated C-3 (3.2 μmol) was digested with α-chymotrypsin (Worthington Biochemical Corp.) at pH 8.0 using conditions and procedures identical with those for trypsin digestion.

Reduced and radioalkylated C-3 (0.65 μmol) was digested at pH 8.0 with thermolysin (Calbiochem) by the addition of 50 μg of enzyme followed by a 2-hr incubation at 37°. The solution was readjusted to pH 8.0 and another 50-μg aliquot of enzyme was added, followed by a 2-hr incubation at 52°.

Chymotryptic and thermolytic digests of tryptic peptide T25 were prepared in the same manner as described above for digests of C-3.

Peptide Purifications. Enzymatically derived peptides were often purified by preparative high-voltage paper electrophoresis using Whatman No. 3MM paper and pH 3.6 and pH 6.5 pyridyl–acetate buffers under Isopar (Esso) in a Savant apparatus as described (Crumpton and Wilkinson, 1965).

A chymotryptic digest of C-3 was first partially resolved by fractionation on a column of Dowex 50-X8 (AA-15 resin, Beckman Instruments) using pyridyl–acetate buffers. Peptides bound to the column equilibrated in 0.05 M pyridyl–acetate (pH 2.4) (0.05 M in pyridine) were eluted with a linear gradient (750 ml/chamber) of pyridyl–acetate buffers (0.05 M in pyridine (pH 2.4) to 1.0 M in pyridine (pH 4.0)). A portion (13%) of the column effluent was continuously monitored in a Technicon Auto Analyzer after reaction with ninhydrin following alkaline hydrolysis.

Sequencing Techniques. Many peptides were subjected to automated Edman degradations using a Beckman Model 890 sequencer. The peptide program (032671, Beckman Instruments) was primarily used to sequence peptides of 6–34 residues. This system employs dimethylallylamine as a solvent buffer. Occasionally, the protein program (Quadrol double cleavage, Beckman Instruments) was used. The thiazolinone derivatives were converted to the phenylthiohydantoin (PTH) derivatives by treatment at 80° for 10 min with 0.2 ml of 1 N HCl, followed by extraction of the derivatives into ethyl acetate. The PTH derivatives were either analyzed directly by gas chromatography on a Beckman GC-45 instrument (Pisano and Bronzert, 1969), or by regeneration of the amino acid by HCl hydrolysis at 140° for 24 hr (Van Orden and Carpenter, 1964). Occasionally, the lysyl residues of small peptides were converted to negatively charged derivatives by the use of 4-sulfophenyl isothiocyanate (Braunitzer *et al.*, 1971).

Manual Edman degradations of peptides were performed according to Gray (1967). The residues removed were iden-

[1] Abbreviations used are TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; PTH, phenylthiohydantoin.

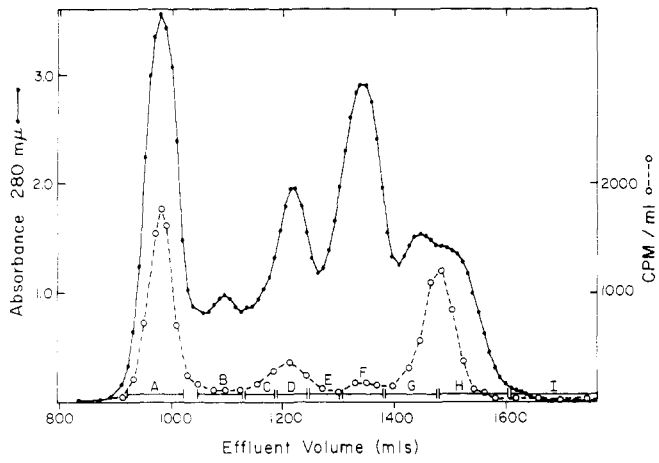

FIGURE 1: Elution profile of a tryptic digest of γ_2 C-3 (3.4 μmol) from a column of Sephadex G-50F in 0.05 M NH_4HCO_3. Column size was 3.0 × 182 cm. Fraction size was 9.7 ml. Radioactivity derives from total reduction and ^{14}C carboxymethylation of C-3.

tified either by gas chromatography as above or by inspection of the amino acid composition of the shortened peptide.

Identification of the amino- or carboxyl-terminal residues of many peptides was achieved by the use of exopeptidases. Carboxypeptidase A (Worthington Biochemical Corp.) digestion was performed at room temperature by the addition of 50 μg of enzyme (1 mg/ml in 1 M NH_4HCO_3) to ~0.02 μmol of peptide. Portions of the digestion mixture were removed at various times, diluted with pH 2.2 citrate buffer to stop the reaction, and analyzed directly on an amino acid analyzer. Carboxypeptidase C (Zuber, 1968) (Rohm and Haas) was dissolved in 0.05 M sodium citrate buffer (pH 5.3) to a concentration of 1000 U/ml and used (5 μl/0.02 μmol of peptide) to digest peptides at pH 5.3 in 0.1 M sodium citrate buffer at 30°. Aliquots were removed and analyzed as described above. Aminopeptidase M (Rohm and Haas) was used in similar fashion in 0.1 M sodium phosphate buffer (pH 7.0) at 37° using 50 μg of enzyme/0.02 μmol of peptide.

Detection of Amino Acids. Amino acid analyses were performed on Beckman Models 120-B and -C amino acid analyzers. Hydrolysis of peptides was carried out in 6 N HCl for 18 hr at 110° *in vacuo* as described previously (Birshtein *et al.*, 1971a). Certain amino acid side chains were detected on electrophoretograms according to Easley (1965). Whether Asx or Glx in peptides was present as the free acid or amide was deduced from the mobility of the peptide at pH 6.5.

Succinylation of Lysine Residues. Reduced and carboxymethylated C-3 (3.0 μmol) was dissolved in 5 ml of 0.01 M phosphate buffer (pH 8); 0.5 mCi of [^3H]succinic anhydride (1 Ci/g) in 0.2 ml of dioxane was added to the solution, followed by the slow addition of 50 mg of solid succinic anhydride. The pH was kept at 8 by addition of 1 M NaOH.

Nomenclature. The numbering of tryptic peptides consecutively from the carboxyl terminus of the γ_2 chain is continued in this paper as outlined previously (Turner and Cebra, 1971). In addition, each chymotryptic peptide from the γ_2 chain bears a different designation. Peptides derived by use of an enzyme on a tryptic peptide bear the designation of the tryptic peptide followed by the new peptide designation.

Results

Tryptic Peptides. Partial resolution of the peptides in a tryptic digest of reduced and carboxymethylated C-3 was achieved by gel filtration on a column of Sephadex G-50 fine in 0.05 M NH_4HCO_3 (Figure 1). Pool A contained only T25, which bears one of the two half-cystine residues in C-3. The other peptides were purified from pools B–H by preparative high-voltage paper electrophoresis at either pH 3.6 or 6.5. Most of these peptides were also isolated in more purified form by gradient elution from a Dowex 1-X2 column (Birshtein *et al.*, 1971b). The amino acid compositions of the tryptic peptides are given in Table I and their amino acid sequences are shown in Figure 2.

T13 was found to be free homoserine by amino acid analysis without acid hydrolysis. The sequences of peptides T14–22 and T26 were determined by sequential manual Edman degradations.

Sequences of peptides T23 and T24 were determined with the use of an automated sequencer using the peptide program for Edman degradation. All of the carbohydrate in the γ_2 chain appears to be contained in the glycopeptide, T24, as this is the only peptide whose amino acid analysis shows the presence of hexosamines. A blank sequencer step in the middle of this 9-residue peptide indicates that hydrophilic carbohydrate moieties are attached to the released amino acid thiazolinone, thus preventing its removal from the reaction cup by chlorobutane. The amino acid in glycosidic linkage must be Asp, as this is the only amino acid not positioned in T24.

The sequence of T25, a 34-residue peptide, was determined by integration of data obtained by a variety of methods as shown by Figure 2. The first 15 residues were determined by automated sequencer analysis of 0.64 μmol of T25 treated with the Braunitzer reagent, 4-sulfophenyl isothiocyanate, and using the peptide program. A chymotryptic digest of 1.07 μmol of T25 was resolved into component peptides by pH 6.5 paper electrophoresis. The compositions of two of these peptides, Ch-T25-1 and Ch-T25-2, are given in Table II. The sequence of Ch-T25-1 was determined with a single Edman degradation and a 10-min carboxypeptidase A digestion. Automated sequencer analysis with the peptide program provided a partial sequence of Ch-T25-2 (0.05 μmol).

The remaining positions in T25 were determined with peptides derived from a thermolytic digest of 1.07 μmol of T25, and resolved by pH 3.6 paper electrophoresis. Their compositions are given in Table III, and their placement within T25 is shown in Figure 2. Partial sequence of Th-T25-3 was established by amino acid analysis of aliquots of a digestion mixture containing aminopeptidase M taken at 10 min and 2 hr. Peptide Th-T25-6 was completely sequenced (0.32 μmol) by automated sequencer analysis using the peptide program.

The alignment of the 14 tryptic peptides of C-3 was accomplished by isolation and analysis of peptides from chymotryptic and thermolytic digests of whole C-3, as well as from a tryptic digest of succinylated C-3.

Chymotryptic Peptides. Reduced and carboxymethylated γ_2 C-3 (3.2 μmol) was digested with chymotrypsin and initial separation of peptides was achieved on a column of Dowex 50-X8 eluted with a gradient of pyridine–acetate buffers (Figure 3). Peptides were further purified from pools 1–15 by pH 3.6 or 6.5 paper electrophoresis. The compositions of peptides overlapping some of the tryptic peptides are given in Table II, and their placement within

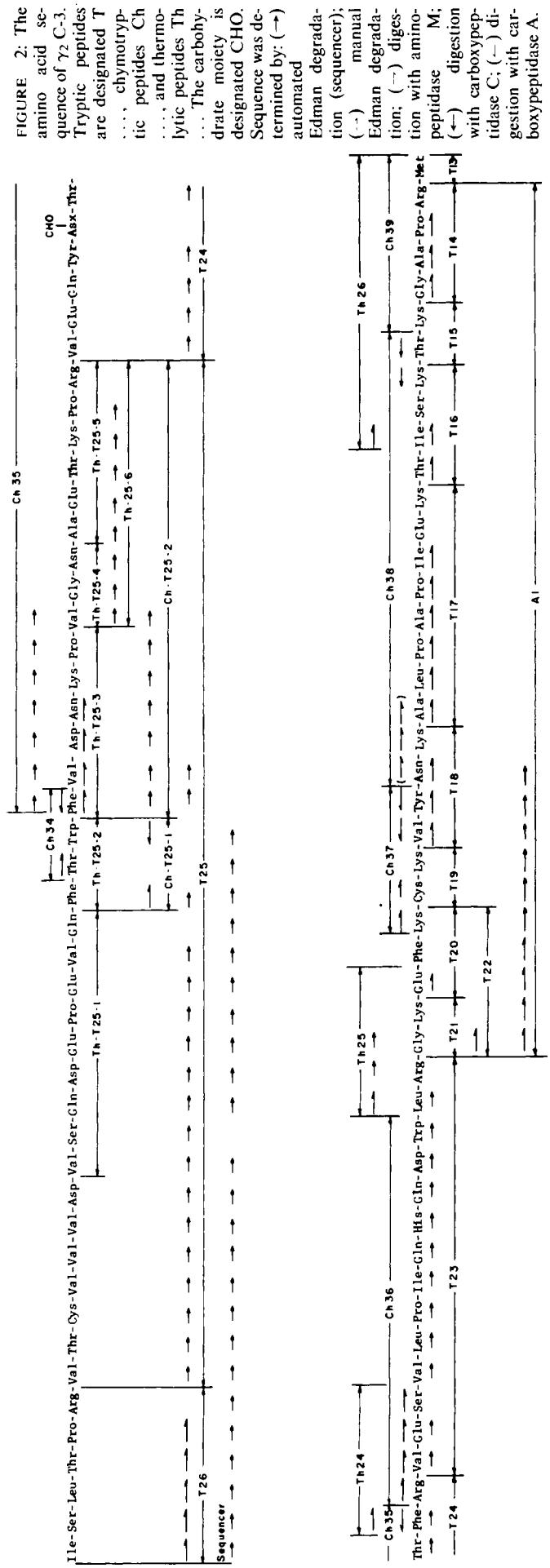

FIGURE 2: The amino acid sequence of γ_2 C-3. Tryptic peptides are designated T ..., chymotryptic peptides Ch ..., and thermolytic peptides Th .... The carbohydrate moiety is designated CHO. Sequence was determined by: (→) automated Edman degradation (sequencer); (–) manual Edman degradation; (–) digestion with aminopeptidase M; (↔) digestion with carboxypeptidase C; (–) digestion with carboxypeptidase A.

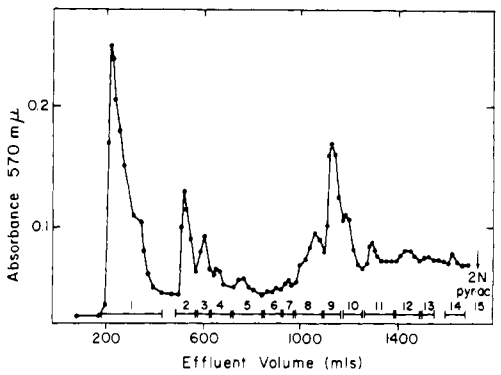

FIGURE 3: Elution profile of a chymotryptic digest of γ_2 C-3 (3.2 μmol) from a column of Dowex 50-X8 eluted with a pyridine-acetate gradient. The column was equilibrated in 0.05 M pyridine-acetate (pH 2.4). After the initial peak emerged, a linear gradient of 0.05 M, pH 2.4 to 1.0 M, pH 4.0 pyridine-acetate (750 ml/chamber) was applied. Finally, elution was carried out with 50 ml of 2 M pyridine-acetate (pH 5.0). The column effluent was divided such that 13% was reacted with ninhydrin after alkaline hydrolysis and absorbance read at 570 mμ. Column size was 0.9 × 57 cm. Fraction size was 9.8 ml.

C-3 is shown in Figure 2. Peptide Ch34 derives from T25 and was sequenced by a single Edman degradation and use of carboxypeptidase A.

The low content of phenylalanine in Ch35, as well as in Ch-T25-2 from T25, was reflected in a double amino-terminal residue. Automated sequencer analysis of Ch35 (and Ch-T25-2) shows two residues at each position, in approximately equimolar amounts. Thus step 1 was found to be Phe, Val; step 2 was Val, Asp, etc., such that the assumption that chymotrypsin splits some molecules between Phe and Val allows a partial sequence of Ch35 to be determined. This peptide contains carbohydrate and exhibits the expected electrophoretic heterogeneity as it was isolated from five distinct positions on paper, each yielding a peptide having an amino acid composition identical with the others. The carboxyl terminus was found to be phenylalanine by carboxypeptidase A digestion indicating that a cleavage at the tyrosyl residue in T24 was probably prevented by the adjacent carbohydrate on the aspartyl residue.

Peptides Ch36, Ch37, and Ch38 were partially sequenced by Edman degradation and digestion with aminopeptidase M or carboxypeptidase A or C. The carboxyl-terminal peptide, Ch39, was isolated in very low yield as it is very basic and is held tightly by Dowex 50. However, its composition, including homoserine, indicates that its placement is correct.

Cleavage at Arginine Residues. As it was suspected that many of the small, lysine-containing tryptic peptides in γ_2 C-3 were derived from the carboxyl-terminal third of the fragment, a single overlap peptide was sought. Reduced and carboxymethylated C-3 (3.0 μmol) was succinylated with [^{3}H]succinic anhydride in order to block and mark lysine residues. Trypsin, which does not cleave adjacent to succinyllysyl residues, was used to cleave at arginyl residues only. Large peptides were isolated on Sephadex G-50 fine in 0.05 M NH_4HCO_3 as shown in Figure 4a. Pool B was found to correspond in composition to T25. Pool A was applied to a column of DEAE-Sephadex A-25 in 0.2 M NH_4HCO_3, developed with a linear gradient 0.2–1.0 M NH_4HCO_3 as shown in Figure 4b. Pool C from this column was rich in lysine and is designated peptide A1. Its composition is given in Table III. Pools A and B were identical with each other and appeared to be comprised mainly of A1. The contaminant in these pools was suspected to be T25, by its high va-

TABLE 1: Tryptic Peptides from γ_2 C-3.[a]

	C-3	T26	T25	T24	T23	T22	T21	T20	T19	T18	T17	T16	T15	T14	T13
Lys	8.5		2.1			3.1	1.1	1.1	1.0	1.3	1.2	1.1	1.1		
His	1.2				1.1									1.1	
Arg	4.3	1.2	1.2	0.94	1.0				0.70						
CM-Cys	2.0		1.1							1.0					
Asp	8.5		5.3	1.1	1.0							0.75	0.67		
Thr	7.9	0.78	2.3	1.9								0.91			
Ser	5.4	0.84	1.0		1.1						1.1				
Glu	11.6		5.1	2.2	2.9	1.0		1.0			2.0			0.96	
Pro	7.4	1.0	3.0		1.0									0.96	
Gly	4.5		1.0			0.95	0.89				2.0			1.1	
Ala	4.3		0.96							0.83					
Val	10.7		6.3	0.91	1.8						1.0	1.0			
Ile	3.3	0.94			0.92						1.0				
Leu	4.6	1.0			1.8					0.81					
Tyr	2.9			2.5											
Phe	5.0		1.9	1.1		1.0		1.0							1.0
Hsr	0.84														
Trp	+		+		+										
CHO	+			+											
Mobility[b]															
pH 3.6		+0.49		−0.26	+0.31	+0.84	+0.95	+0.49	+0.30	+0.57	+0.41	+0.62	+0.84	+0.71	−0.18
pH 6.5		+0.43		−0.07	−0.01	+0.52		+0.04	−0.05	+0.50	+0.05	+0.55	+0.72	+0.65	−0.05
Pool (Figure 1)		G	A	B	F	G	G,H	H	G,H	H	E,F	G	G,H	G,H	H

[a] The values in this and subsequent tables represent moles of a particular amino acid per mole of peptide. [b] Mobility given relative to Lys = +1.0, Asp = −1.0, and neutral amino acids = 0.

TABLE II: Chymotryptic Peptides from γ_2 C-3 and T25.

	T25		C-3					
	Ch-T25-1	Ch-T25-2	Ch34	Ch35	Ch36	Ch37	Ch38	Ch39
Lys		2.2		1.8	0.11	2.1	3.1	0.93
His					1.0			
Arg		1.1		1.2	1.1			1.1
CM-Cys						0.71		
Asp		3.1		3.9	1.0		1.1	
Thr	0.81	1.3	0.86	2.6			1.6	
Ser					0.97		0.81	1.4
Glu		1.0		3.0	3.0		1.2	
Pro		2.2		2.1	1.0		2.7	0.64
Gly		0.98		1.1		0.29		1.8
Ala		0.96		1.1		0.39	2.3	1.0
Val		2.1		2.9	2.0	1.0		
Ile					0.96		1.9	
Leu					1.0		1.3	
Tyr				0.90		0.77		
Phe	1.0	0.46	1.0	1.5				
Hsr								+
Trp	+		+		+			
CHO				+				
Mobility								
pH 3.6	+0.11	+0.49	+0.23	+0.16	+0.40	+0.63	+0.68	+0.95
pH 6.5	−0.02	+0.03	0	−0.02	0	+0.34		
Pool (Figure 3)			10	1,2	9	15	8	15

TABLE III: Thermolytic Peptides from γ_2 C-3 and T25 and a Tryptic Peptide from Succinylated γ_2 C-3.

	T25						C-3			
	Th-T25-1	Th-T25-2	Th-T25-3	Th-T25-4	Th-T25-5	Th-T25-6	Th24	Th25	Th26	A1
Lys			1.0		1.0	1.0		1.0	2.0	7.8
Arg					1.2	1.0	(1)	0.97	1.0	+
CM-Cys										+
Asp	1.1		2.0	0.63		1.1				1.7
Thr		0.92			0.92	0.95			0.76	1.9
Ser	1.5						(1)		0.76	1.5
Glu	3.9				1.1	1.1	(1)	1.2		2.5
Pro	0.99		1.0		(1)	0.97			+	2.6
Gly				1.0		1.1		1.1	1.1	1.8
Ala					0.81	1.0			1.1	1.5
Val	1.9		0.88	1.2		0.93	(1)			1.2
Ile									0.71	1.6
Leu								0.85		1.0
Tyr										0.92
Phe	0.28	0.66	0.66				(1)			1.1
Hsr									+	
Trp		+								
Mobility										
pH 3.6	−0.17	+0.11	+0.42	+0.29	+0.77	+0.66	+0.46	+0.82	+0.82	
pH 6.5		0					0	+0.49	+0.82	

line and aspartic acid content. A single Edman degradation of A1 (pool C, Figure 4b) showed that Gly is the amino terminus. A single Edman degradation of the peptide(s) in pools A and B (Figure 4b) showed Gly and some Val, as would be expected from contaminating T25. As the sequence of T25 was known, pools A, B, and C were combined for further Edman degradations, three of which were done manually and the next six were done by automated degradation using the peptide program. The apparent charge heterogeneity in A1, leading to its isolation in different pools from an ion exchange column, might be due to incomplete succinylation of some of its seven lysyl residues.

Thermolytic Peptides. In order to rigorously align the γ_2 C-3 tryptic peptides T24-T23, T23-T22, and T16-T15-

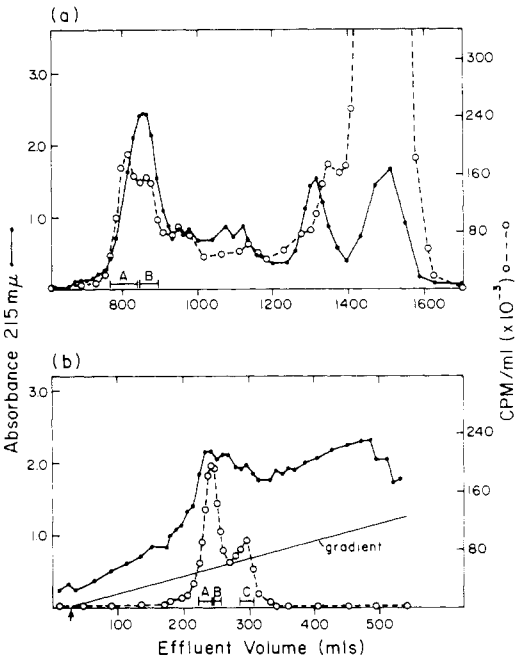

FIGURE 4: (a) Elution profile of a tryptic digest of ^{3}H-succinylated γ_2 C-3 (3.0 μmol) from a column of Sephadex G-50F in 0.05 M NH_4HCO_3. Column size was 3.2 × 182 cm. Fraction size was 7.7 ml. (b) Elution profile of the components in pool A, Figure 4a, from a column of DEAE-Sephadex A-25 eluted with an NH_4HCO_3 gradient. The column was equilibrated in 0.2 M NH_4HCO_3 and was eluted with a linear gradient of 0.2–1.0 M NH_4HCO_3 (250 ml/chamber). Column size was 1.0 × 13 cm. Fraction size was 4.5 ml.

T14-T13, three arginine-containing thermolytic peptides were sought. A thermolytic digest of 0.65 μmol of reduced and carboxymethylated C-3 was partially resolved by pH 3.6 paper electrophoresis. Arginine peptides, identified by the Sakaguchi stain, were eluted and each peptide mixture was subsequently purified by pH 6.5 paper electrophoresis. Peptides Th24, Th25, and Th26, whose compositions are given in Table III, were among the arginine-positive peptides in the second purification step. Peptide Th24 appeared to be contaminated by the peptide Val-Asp-Asn-Lys-Pro, which is derived from T25 and is a shorter analog of Th-T25-3 from the T25 thermolytic digest. The fact that both Th24 and its contaminant were electrophoretically neutral at pH 6.5 is additional indication that the contaminating peptide is the Th-T25-3 analog. A single Edman degradation of this mixture yielded equimolar amounts of Phe and Val. Thus, Th24 has an amino-terminal Phe. The suggested composition of Th24 given in Table III was gotten by subtracting from the composition of the mixture, the known composition of peptide Th25. Edman degradations of peptides Th25 and Th26 confirmed their placement by composition alone, as shown in Figure 2.

Automatic Sequencer Analysis of C-3. Alignment and sequence confirmation of peptides T26 and T25 at the amino-terminus of γ_2 C-3 were accomplished by automated sequencer analysis of 2.0 μmol of reduced and carboxymethylated C-3 using the protein program. As seen in Figure 2, only one step remained unidentified in the 25 steps analyzed, and this corresponded to a serine from T25 which is difficult to determine by this procedure. An attempt to purify the residual C-3 after 25 degradative steps by filtration through Sephadex G-50 fine in 0.05 M NH_4HCO_3 resulted in apparent blockage of the new amino terminus as no further information was obtained by automated sequencer analysis of the shortened C-3.

Discussion

The primary structure of a 93-residue cyanogen bromide fragment, C-3, from the Fc region of guinea pig γ_2 chain has been determined and the many complementary data for the peptides obtained from various digests were consistent with C-3 having a single amino acid sequence. The se-

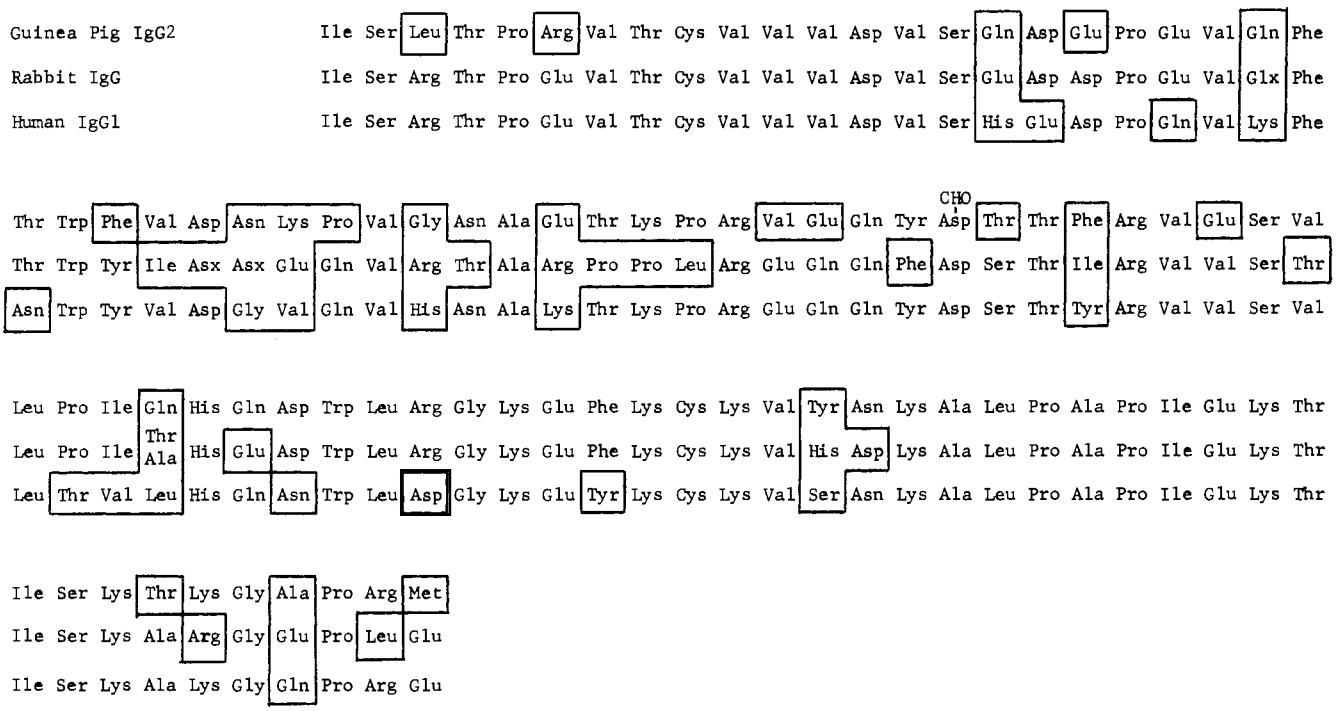

FIGURE 5: A comparison of the amino acid sequence of γ_2 C-3 with the sequences in analogous regions of rabbit IgG (Hill *et al.*, 1967) and human IgG1 (Edelman *et al.*, 1969). A closed rectangle is placed around each residue at a given position which is present in only one species. For purposes of comparison, Asx and Glx are considered nonidentical with the corresponding acid or amide.

quence determined for guinea pig γ_2 chain between residues ~N-249 and ~N-341 can be compared with corresponding sections from rabbit IgG (Hill *et al.*, 1967; Appella *et al.*, 1971) and a human IgG1 myeloma protein, Eu (Edelman *et al.*, 1969), as shown in Figure 5. A great deal of strict homology of sequence (~69%) between species is observed in this region (65 residues identical at 93 residue positions compared between guinea pig γ_2 and human γ_1, 63/93 between guinea pig γ_2 and rabbit γ_1, and 64/93 between rabbit γ and human γ_1). It is striking that the region spanned by residues ~N-322 and ~N-334 is completely conserved among these species.

It may eventually be possible to correlate the amino acid sequences corresponding to guinea pig γ_2 C-3 with some biologic functions of the parent molecules, notably the initiation of the activation of complement *via* the conventional pathway. All three IgGs whose partial sequences are shown in Figure 5 are capable of participating in complement fixation. However, it would be naive to speculate about particular amino acids which may be involved in the initiation of this process because nothing of the tertiary structure in this region of IgG is known.

The suggestion has been made that regions in immunoglobulin molecules are folded into compact globular structures called domains, each of which may be responsible for particular biologic activities of the molecule (Edelman *et al.*, 1969). The Fc fragment of IgG molecules is comprised of two homology regions, C_H2 and C_H3, symmetric dimers of which may form functional domains (Poljak *et al.*, 1972).

There is good evidence that the amino-terminal section of the C_H2/C_H2 domain is the area within Fc to which the first component of complement, C'1q, binds. Selective removal of the C_H3/C_H3 domain of rabbit IgG by cleavage with plasmin does not affect the ability of IgG to initiate complement fixation (Connell and Porter, 1971). In addition, direct initiation of complement fixation by an intact C_H2 product derived from human IgG1 has been demonstrated (Ellerson *et al.*, 1972). A 62-residue CNBr fragment from mouse IgG2a has some complement fixing activity (~6% of whole molecule) and it derives from the C_H2 homology region (Kehoe and Fougereau, 1969). In a careful study, Utsumi (1969) degraded rabbit Fc with papain under varied conditions which removed, in a stepwise fashion, the C_H2 region. Complement fixing activity was retained by a fragment (~46,000 daltons) which lacked the "hinge region," but activity was lost upon degradation to ~40,000 daltons with apparent cleavage of the intrachain disulfide bond within C_H2. Both active and inactive fragments retained the carbohydrate moiety which is attached near the middle of the C_H2 region.

As the cyanogen bromide fragment guinea pig γ_2 C-3 contains an intact disulfide bond spanning the region containing the oligosaccharide, it may retain much of the conformation of the entire C_H2 region of guinea pig IgGs and be susceptible to renaturation to a C_H2/C_H2 dimer. Thus, it may be possible to directly employ fragment γ_2 C-3 for the characterization of the binding sites on IgG for specific interaction with complement component C'1q.

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