Structure of the Heavy Chain from Strain 13 Guinea Pig Immunoglobulin G1: Isolation of Cyanogen Bromide Fragments†

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ABSTRACT: Guinea pig serum contains two isotypes of immunoglobulin G: IgG1 and IgG2. These immunoglobulins are antigenically distinct from each other and they mediate different biologic processes in the same guinea pig, although they share the ability to bind the same antigen. An attempt was made to study the primary structure of the γ_1-heavy chain from IgG1 in comparison with the largely known primary structure of the γ_2-heavy chain from IgG2, with the aim of demarcating the structural differences between these molecules. IgG1 was isolated from the serum of immune strain 13 guinea pigs. Both IgG1 and the γ_1 chain were digested with CNBr. Nine fragments were isolated from both digests by gel filtration procedures before and after reductive cleavage of disulfide bonds. These fragments appear to account for the entire ~444 residues in the γ_1 chain. Amino acid composition data of CNBr fragments suggest that at least the amino terminal ~182 residues of the γ_1 and γ_2 chains are very similar. Two of the fragments which have been isolated have amino acid compositions suggesting their derivation from the "hinge" region and carboxyl terminus of the γ_1 chain.

Although a great deal is known about the structures of immunoglobulins and myeloma proteins, many questions concerning the relationships of their structures to their antigen binding and other biologic activities are still unresolved. One approach toward answering some of these questions is to compare the structures of different classes or subclasses of immunoglobulin from the same outbred individual or from different members of an inbred species.

The heavy and light polypeptide chains comprising immunoglobulins are each thought to be derived from two genes which code for the variable (V) and constant (C) regions of the polypeptide chains (Dreyer and Bennett, 1965; Press and Hogg, 1969; Cunningham et al., 1969). Comparisons of V_H and C_H amino acid sequences of specific antibodies of different isotypes may permit inferences concerning the genetic basis for the generation of antibody diversity. In addition, such comparisons may indicate structural differences between immunoglobulin isotypes which correlate with differences in biologic properties such as complement activation, opsonization, or the ability to mediate allergic reactions. Eventually, one would hope to be able to define in structural terms the basis for those specific interactions with complement component Clq, monocyte bound Fc receptor, or mast cell Fc receptor which respectively initiate each of these biologic phenomena.

An appropriate set of antibodies to use in an attempt to correlate heavy chain C region structures with the activities of the parent molecules would be those of different isotypes from an inbred species. Thus, pooled immunoglobulins of a given isotype from different individuals should share identical C regions combined with a mixture of V regions reflecting the different antibody specificities of the parent molecules. A unique opportunity to study such heavy chain isotypes is provided by the presence of relatively large amounts of two subclasses of IgG in the serum of inbred guinea pigs. The IgG1 and IgG2 subclasses in guinea pig serum are well defined serologically and with respect to their biologic activities. These subclasses can be recognized and purified on the basis of their electrophoretic differences (Benacerraf et al., 1963; White et al., 1963; Oettgen et al., 1965; Oliveira et al., 1970).

The complete primary structure of the γ_2 chain of normal IgG2 from Wright strain 13 guinea pigs has nearly been elucidated in our laboratory. The entire γ_2 chain can be accounted for by eight cyanogen bromide fragments which have been isolated (Birshtein et al., 1971; Birshtein and Cebra, 1971) and aligned (Benjamin et al., 1972). In addition, primary structural data obtained from the variable regions of antibodies of three different specificities (Cebra et al., 1974) have provided information concerning the residue positions of amino acids involved in the antigen-binding site.

Using the basic strategy developed for the amino acid sequence analysis of the γ_2 chain, the isolation and alignment of cyanogen bromide fragments of the γ_1 chain were undertaken. This manuscript reports the isolation and compositions of nine cyanogen bromide fragments which appear to account for the entire γ_1 chain.

Experimental Procedure

Immunization of Animals. Wright strain 13 guinea pigs were immunized with Dnp-KLH,[1] which was prepared according to Eisen (1964). The following immunization regimen (Asherson and Stone, 1965) was chosen because it results in a deviation of the normal immune response to hapten–protein conjugates such that the serum IgG1 antibody level is greatly increased relative to the level of IgG2 antibody. Twelve to eighteen animals were each injected with 1 mg of Dnp-KLH in alum-precipitated form. The suspension of antigen was distributed among four footpad sites (0.15

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[1] Abbreviations used are: Dnp, dinitrophenyl; KLH, keyhole limpet hemocyanin; CMCys, carboxymethylcysteine; Hsr, homoserine.

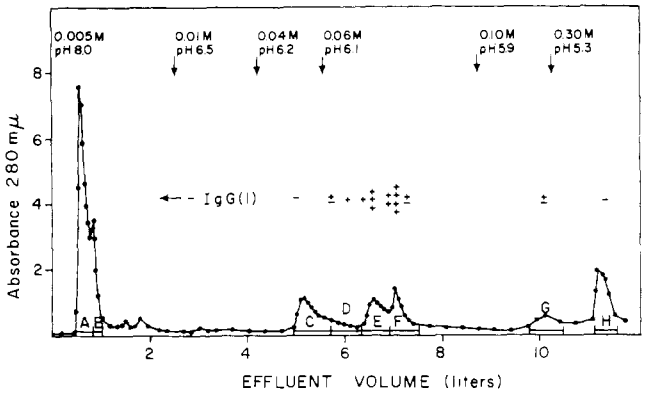

FIGURE 1: Elution profile of the "pseudoglobulin" fraction of immune guinea pig serum from a column of Whatman DE-52 cellulose with phosphate buffers. The column (5.0 × 60 cm) was equilibrated in 0.005 M sodium phosphate (pH 8.0) and proteins were eluted in a stepwise fashion using 0.01 M, pH 6.5; 0.04 M, pH 6.2; 0.06 M, pH 6.1, 0.10 M, pH 5.9; and 0.30 M, pH 5.3 sodium phosphate buffers. Fraction size was 16.8 ml. Occasional tubes were screened for IgG1 content by gel diffusion analysis with a specific rabbit anti-gunea pig IgG1 antiserum, and precipitin lines were scored from − to ++++.

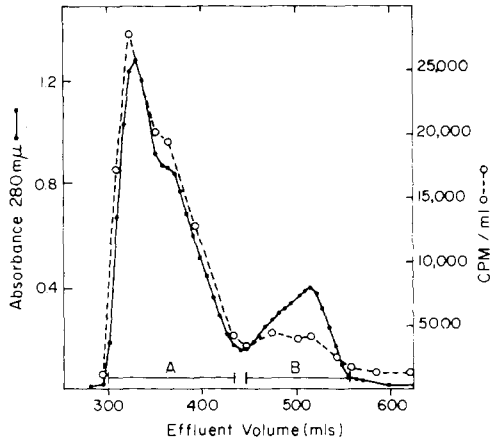

FIGURE 2: Elution profile of the heavy and light chains of IgG1 from a column of Sephadex G-75 in 1.0 M propionic acid after mild reduction and ^{14}C-carboxymethylation of IgG1. Column size was 2.1 × 225 cm. Fraction size was 7.1 ml.

ml/site) and four intradermal sites (0.1 ml/site), two on each flank. After 14 days the animals were injected in the footpads with 100 μg of Dnp-KLH emulsified in complete Freund's adjuvant. After 14 days the animals were bled (~8 ml) by cardiac puncture four times at 4-day intervals and finally bled to death.

Alternatively, the immune response was deviated toward increased IgG1 antibody production by carrier priming. This was accomplished as described by Liu et al. (1974) by primary immunization with KLH followed by a secondary immunization with Dnp-KLH.

Preparation of IgG1. The pooled serum (330 ml) from alum/antigen primed animals was brought to 40% saturation with ammonium sulfate. The washed globulin precipitate was dissolved in saline (60 ml) and exhaustively dialyzed vs. 0.005 M sodium phosphate (pH 8.0) in the cold. The pseudoglobulin, or supernatant fraction after dialysis and centrifugation, was chromatographed on a large column of Whatman DE-52 cellulose by a procedure modified from Oliveira et al. (1970). The column was initially equilibrated in 0.005 M sodium phosphate (pH 8.0) and was eluted at room temperature in a stepwise manner with sodium phosphate buffers that were 0.01 M, pH 6.45; 0.04 M, pH 6.2; 0.06 M, pH 6.1; 0.10 M, pH 5.9; and 0.30 M, pH 5.3. A typical elution profile is shown in Figure 1.

Selected tubes were screened for IgG1 content using a specific rabbit anti-guinea pig IgG1 antiserum. Only pools D–F had significant activity. Each of the pools D–F was further purified by gel filtration on Sephadex G-200, where IgG1 always represented the major component. This material appears pure by immunoelectrophoretic analysis developed with a rabbit anti-guinea pig serum.

The immunoglobulin yields from these purification procedures are variable, but are approximately 1.8 mg/ml of serum for IgG1 and 3.7 mg/ml of serum for IgG2 (pools A and B, Figure 1).

Purified anti-Dnp IgG1 was prepared as described by Liu et al. (1974) from serum of carrier-primed animals for direct digestion of intact antibodies with CNBr.

Isolation of γ_1 Chain. IgG1 (100 mg) was mildly reduced with 0.01 M dithiothreitol and subsequently carboxymethylated with 0.02 M iodoacetic-*1*-^{14}C acid as described by Birshtein et al. (1971) in order to mark cysteinyl residues which had participated in the formation of labile disulfide bonds. Heavy and light chains were then separated on a column of Sephadex G-75 in 1.0 M propionic acid, whose elution profile is shown in Figure 2. The ratio of the number of labeled half-cystines in heavy and light chains (pools A and B, respectively) was determined to be 5.2:1 based on published molecular weights and extinction coefficients (Leslie and Cohen, 1970) and the specific activity of the iodoacetic-*1*-^{14}C acid.

Cleavage of IgG1 and γ_1 Chain with CNBr. Lyophilized IgG1 (350 mg) or γ_1 chains (Figure 2, pool A, 58 mg) were digested with CNBr in 70% formic acid at 5 °C for 24 h as described by Birshtein et al. (1971). The reaction was terminated by the addition of 10 volumes of water per volume of reaction mixture and immediately frozen and lyophilized to remove excess reagents. For the initial fractionation of CNBr fragments, this material was dissolved in about 5 ml of 8 M urea, 0.1 M in formic acid, and applied directly to a Sephadex G-100 column equilibrated in the same solvent.

Total Reduction and Alkylation of Cysteinyl Residues in Disulfide Bonds. Cleavage of all disulfide bonds in a protein or peptide was carried out as described by Birshtein et al. (1971). Briefly, lyophilized protein or peptide was made 5–10 mg/ml in 7 M guanidine hydrochloride (ultrapure, Mann Research Laboratories) made 0.1 M in Tris-acetate (pH 8.0) and reduced with 0.05 M dithiothreitol. Carboxymethylation of the resulting cysteinyl residues was carried out with 0.11 M iodoacetic acid, often containing iodoacetic-*1*-^{3}H acid (0.3 mCi). The solutions were applied immediately to an appropriate Sephadex column for fractionation of component peptides.

Amino Acid Analyses. Amino acid analyses were performed on Beckman Models 120 A, B, and C amino acid analyzers using Dowex AA-15 resin. Samples (~0.01 μmol of peptide) were hydrolyzed in 0.5 ml of constant-boiling HCl containing 10 μl of 0.1 N phenol and 10 μl of 0.05 M dithiothreitol in sealed, evacuated Pyrex tubes immersed in a boiling toluene bath (110 °C) for 18 h. The hydrolysates were evaporated to dryness. If homoserine was suspected, the homoserine lactone was converted to free homoserine by heating the dried hydrolysate at 100 °C for 30 min in 0.5 ml of pyridine–acetate buffer (10% pyridine) (pH 6.5) in a stoppered tube (Ambler, 1965).

The presence of carbohydrate in a sample was judged by

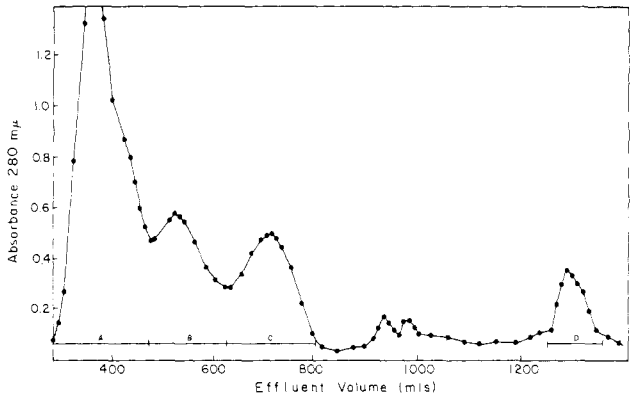

FIGURE 3: Elution profile of a CNBr digest of intact IgG1 from a column of Sephadex G-100 in 8 M urea, 0.1 M in formic acid. Column size was 3.5 × 250 cm. Fraction size was 10 ml.

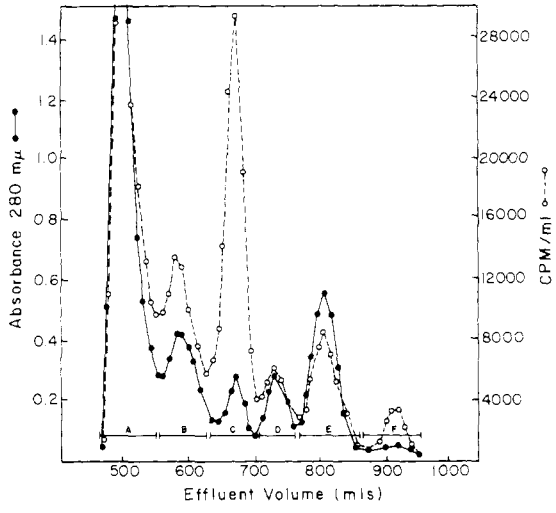

FIGURE 4: Elution profile of the components in pool A, Figure 3, after total reduction and ^{3}H-carboxymethylation, from a column of Sephadex G-50F in 8 M urea, 0.1 M in formic acid. Column size was 3.5 × 190 cm. Fraction size was 9.5 ml.

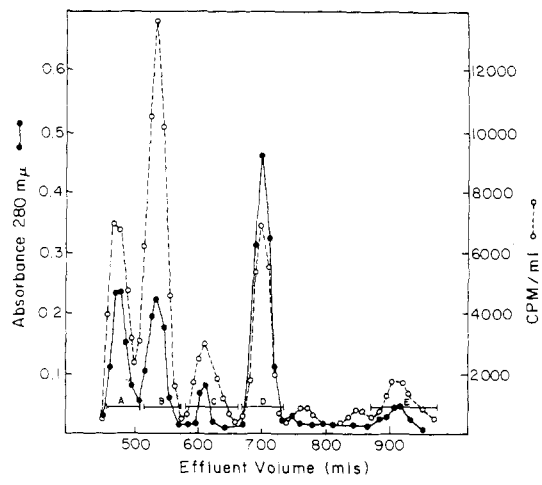

FIGURE 5: Elution profile of the components in pool C, Figure 3, after total reduction and ^{3}H-carboxymethylation, from a column of Sephadex G-50F in 8 M urea, 0.1 M in formic acid. Column size was 3.5 × 190 cm. Fraction size was 9.7 ml.

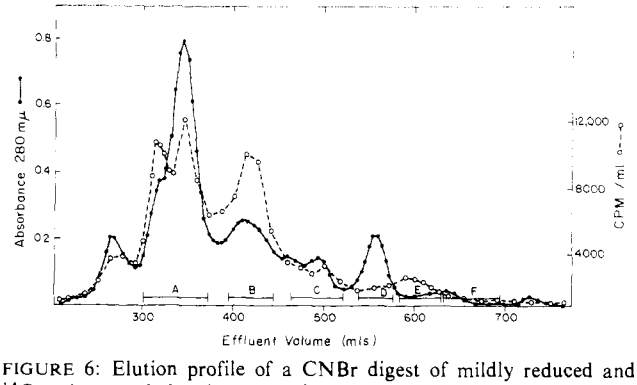

FIGURE 6: Elution profile of a CNBr digest of mildly reduced and ^{14}C-carboxymethylated γ_1 chain from a column of Sephadex G-100 in 8 M urea, 0.1 M in formic acid. The sample corresponds to the material in pool A, Figure 2. Column size was 2.1 × 225 cm. Fraction size was 4.6 ml.

the hexosamine peaks on long and short column amino acid analyses.

Other Procedures. Urea (8 M) for column chromatography was freed of electrolytes by passage through a column of Amberlite MB-3.

The absorbance of protein solutions and column effluents was measured in a Zeiss M4QIII spectrophotometer. Absorbance in NH_4HCO_3 or NH_4OH effluents was measured at 215 mμ, formic acid effluents were monitored at 230 mμ, and all other effluents were monitored at 280 mμ. Extinction coefficients used for protein solutions were those published by Leslie and Cohen (1970): $E_{280\,m\mu}$ (1%) = 15.2, IgG1; 14.7, IgG2; 15.9, γ_1 chain; 15.6, γ_2 chain; 12.8, L chains.

Radioactivity of 50–100-μl samples was measured in a liquid scintillation counter (Packard TriCarb or Nuclear Chicago) in glass vials with 5 ml of scintillation fluid (Kinard, 1957).

Pools of column effluents containing urea were desalted on columns of Sephadex G-25C equilibrated in 0.05 M formic acid. The formic acid was then removed by freeze drying. Pools of column effluents containing NH_4HCO_3 or NH_4OH were freeze dried to remove these salts.

Nomenclature. The cyanogen bromide fragments derived from γ_1 chain are designated in a fashion analogous to those derived from the γ_2 chain (Birshtein et al., 1971; Birshtein and Cebra, 1971). In the γ_2 chain, the fragments are aligned (Benjamin et al., 1972) from the amino to the carboxyl terminus as follows: C-1-n, C-1-a_1, C-1-a_2, C-1-b, C-1-c, C-3, C-4, and C-5. Fragments isolated from γ_1 chain which appear very similar to γ_2-chain fragments, as judged by amino acid compositions and gel filtration elution volumes, are given the same designations as their γ_2 analogues. However, in order to avoid confusion, all fragments referred to hereafter will be preceded by the chain type from which the fragment derived: e.g., γ_1C-1-n, γ_2C-1-n. We have found that the region in the γ_1 chain analogous to γ_2C-3/C-4 appears to be represented by three CNBr fragments, so these have been designated γ_1C-2, γ_1C-3, and γ_1C-4 (D. Tracey and J. Cebra, unpublished data).

Results

Isolation of CNBr Fragments from Whole IgG1. The isolation of CNBr fragments is based on successive gel filtrations before and after reductive cleavage of disulfide bonds that were interchain or intrachain bonds in the intact molecule. The initial fractionations involve separation of fragments into various size groups, some of which contain several fragments linked by disulfide bonds. The identities and purities of fragments isolated by these procedures are largely based on amino acid compositions and on elution volumes from calibrated columns of Sephadex equilibrated

Table I: Amino Acid Compositions of Cyanogen Bromide Fragments from γ_1 and γ_2 Chains.[a]

	C-1-n		C-1-a_1		C-1-a_2		C-1-b		C-1-c		C-2	C-3	C-4	Σ[b]	C-3, C-4	C-5	
	γ_1	γ_2	γ_1	γ_2	γ_1	γ_2	γ_1	γ_2	γ_1	γ_2	γ_1	γ_1	γ_1	γ_1	γ_2	γ_1	γ_2
Lys	0.53	0.7	2.6	3.0	1.5	2.1	2.0	2.6	6.2	7.7	1.5	6.8	2.2	10.5	15.5	1.2	1.0
His			0.35		0.55	0.6	0.79	0.85	2.0	(1)	0.40	1.1		1.5	1.9	2.1	2.5
Arg	0.79	1.0	2.7	2.9	2.2	3.0	0.58	0.30		0.72	0.76	3.9	1.3	6.0	6.2	0.95	1.1
CMCys	0.90	0.7			1.6	1.5	1.0	1.1	3.7	3.3	0.98	2.2	1.1	4.3	4.1		
Asp	0.55	1.4	6.0	4.6	5.2	4.6	1.3	1.2	4.7	4.8	2.2	7.0	10.7	19.9	18.2	1.2	1.1
Thr	1.4	1.6	3.3	3.5	5.0	5.0	5.0	4.2	5.6	6.0	2.1	7.8	4.9	14.8	12.4	0.42	1.1
Ser	5.0	4.3	3.2	4.2	7.1	5.9	5.2	4.3	5.4	3.9	3.5	6.0	7.1	16.6	13.0	2.5	2.0
Glu	5.0	4.2	3.9	3.2	3.2	2.7	3.3	2.2	3.9	4.3	3.9	8.7	4.5	17.1	17.4	2.4	2.1
Pro	1.1	1.4	1.4	1.9	3.4	3.5	2.6	3.1	9.9	14.0	1.8	7.4	3.8	13.0	15.5	1.5	0.84
Gly	5.1	5.1	5.8	5.2	4.1	4.2	5.1	4.7	5.7	2.1	3.0	3.5	2.9	9.4	5.8	1.3	1.2
Ala	2.0	1.8	3.2	2.8	4.6	5.3	1.9	2.2	3.6	3.0	1.6	5.2	2.6	9.4	8.5	1.3	1.9
Val	3.0	3.2	2.6	2.8	4.8	4.7	4.4	4.6	6.5	5.6	3.6	5.9	4.1	13.6	20.1	0.88	0.86
Ile			2.8	2.9	1.0	1.6	0.58		3.0	2.1	0.89	3.9	1.9	6.7	7.2	1.3	0.99
Leu	3.9	3.9	2.7	3.5	3.4	3.5	5.3	5.1	3.6	2.3	2.5	9.5	2.1	14.1	9.8	0.98	0.99
Tyr	1.4	1.3	2.3	2.4	2.9	2.9	2.2	1.7			1.2	1.1	2.9	5.2	8.3		
Phe	1.9	1.8	1.6	1.5	2.2	1.9	1.1	1.9	2.1	1.9	1.6	2.8	4.7	9.1	7.0		
Hsr	1.1	1.0	1.5	1.2	0.94	0.9	1.0	1.0	0.99	1.0	0.90	+	1.2	(3)	1.8		
Trp[c]		±		+		+		+		−					+		−
CHO	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	−	−
Total[d]	34	34	49	49	55	55	44	44	66	66	32	84	58	174	178	18	18

[a] Numbers in this table represent moles of a given amino acid per mole of fragment after 18-h hydrolysis in constant boiling HCl. These figures are averages of several analyses from different preparations. The γ_2 analyses are derived from normal γ_2 chain as quoted in the following sources: C-1-n and C-1-a_2, Cebra et al. (1971); C-1-a_1 (normalized to 49 residues), C-1-b, C-1-c, and C-5, Birshtein et al. (1971); C-3, Tracey and Cebra (1974); C-4, Trischmann and Cebra (1974). Blanks for certain amino acids in some fragments indicate negligible quantities present. [b] This column represents the sum of the analyses of γ_1C-2, γ_1C-3, and γ_1C-4. [c] Tryptophan was determined qualitatively by the Ehrlich stain. Blanks indicate that staining was not performed with these fragments. [d] These values are the most probable total lengths of the fragments, including tryptophan residues, as determined by a number of analyses.

in dissociating solvents such as 8 M urea, 0.1 M in formic acid. Such elutions allow an approximation of the size of a fragment. Also, tentative assignment of some γ_1 fragments as analogues of γ_2 fragments can be made based on these criteria which have been established for all the γ_2 fragments (Birshtein et al., 1971; Birshtein and Cebra, 1971).

A CNBr digest of intact IgG1 was initially fractionated on a column of Sephadex G-100 as shown in Figure 3. The material in pool D has an elution volume and amino acid composition very similar to that of the 49-residue fragment γ_2C-1-a_1, and will thus be designated γ_1C-1-a_1 (Table I).

Pool A was expected to contain L chains disulfide bonded to some H chain fragments. Total reduction and radioalkylation of the material in pool A was followed by gel filtration on Sephadex G-50 as shown in Figure 4. All cysteinyl residues which had participated in disulfide bonds are labeled in this procedure. The extremely radioactive fragment eluting in pool C (Figure 4) has an elution volume identical with that of γ_2C-1-c and its amino acid composition (Table I) reveals a very high half-cystinyl and prolyl content, indicating that this is probably the 66-residue "hinge" region fragment γ_1C-1-c. Pools D, E, and F from Figure 4 have elution volumes and amino acid compositions (Table I) very similar to the γ_2 fragments γ_2C-1-a_2, γ_2C-1-b, and γ_2C-1-n, respectively. Thus, the fragments in these three pools are designated 55-residue γ_1C-1-a_2, 44-residue γ_1C-1-b, and 34-residue γ_1C-1-n.

The material in G-100 pool C (Figure 3) was also totally reduced and radioalkylated and passed through a column of Sephadex G-50 as shown in Figure 5. The elution volumes and amino acid compositions of the fragments in pools A–E, Figure 5, correspond to none of the γ_2 chain CNBr fragments. For reasons which will be outlined below, the material in pools B, D, and E correspond to fragments of approximately 84, 58, and 32 residues in length, designated γ_1C-3, γ_1C-4, and γ_1C-2, respectively. The recovery of γ_1C-2 from pool E is variable and never equimolar to γ_1C-3 or γ_1C-4. The possibility exists that γ_1C-2 aggregates to a large extent and elutes at the void volume in pool A.

No attempt was made to recover the presumptive 18-residue carboxyl-terminal fragment, γ_1C-5, from the eluate of the G-100 column (Figure 3). However, the fragments described above could account for the other 430 residues of γ_1 chain. To confirm that the fragments so isolated did arise from the heavy chain, and to detect fragments missed in the whole molecule fractionation, a CNBr digest of purified γ_1 chain was performed.

Isolation of CNBr Fragments of γ_1 Chain. A CNBr digest of mildly reduced and ^{14}C-carboxymethylated γ_1 chain was initially fractionated on a column of Sephadex G-100 as shown in Figure 6. It is striking that most of the peptide material absorbing light at 280 mμ was found in pool A, whereas in an analogous fractionation of γ_2 fragments from a CNBr digest (Birshtein et al., 1971) most of the material eluted in a volume corresponding to pools B and C (Figure 6).

Most of the fragments of the γ_1 chain can be derived from the material in pool A, Figure 6, by total reduction and alkylation followed by filtration on Sephadex G-50 as shown in Figure 7. Pool C (Figure 7) contained most of the radiolabel derived from mild reduction and radioalkylation of IgG1, and its elution volume corresponds to the elution volume from a similar column of the highly labeled "hinge" region fragment, γ_2C-1-c. Thus the material from pool C was identified as γ_1C-1-c, although its amino acid composition is significantly different from that of γ_2C-1-c.

Pools E and F (Figure 7) were found to contain γ_1C-1-b and γ_1C-1-n, respectively, as judged by elution volumes, amino acid compositions corresponding to γ_2C-1-b and γ_2C-1-n (Table I), and lack of radiolabel.

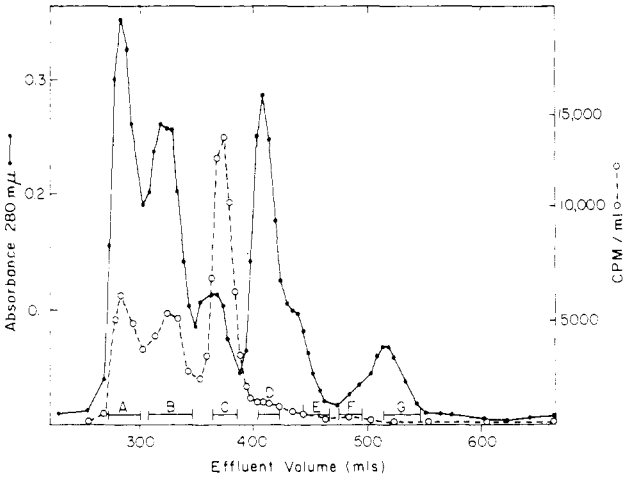

FIGURE 7: Elution profile of components in pool A, Figure 6, after total reduction and carboxymethylation, from a column of Sephadex G-50F in 8 M urea, 0.1 M in formic acid. Radioactivity derives from mild reduction and ^{14}C-carboxymethylation of γ_1 chain. Column size was 2.5 × 150 cm. Fraction size was 5.0 ml.

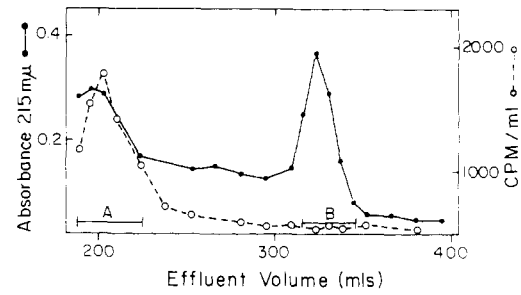

FIGURE 8: Elution profile of components in pool E, Figure 6, from a column of Sephadex G-50F in 0.05 M NH_4OH. Radioactivity derives from mild reduction and ^{14}C-carboxymethylation of γ_1 chain. Column size was 2.8 × 87 cm. Fraction size was 7.2 ml.

As γ_2C-1-n is disulfide bonded to γ_2C-1-a$_2$ in intact γ_2 chain (Ray and Cebra, 1972), it was expected that an amount of γ_1C-1-a$_2$ equimolar to γ_1C-1-n would be found in pool D, Figure 7. The extraordinarily high optical density peak of the fractions eluted at this volume of effluent indicates that another fragment, of approximately 55 residues, is coeluting with presumptive γ_1C-1-a$_2$. Indeed, the amino acid composition of pool D is not similar to γ_2C-1-a$_2$, and it is proposed that γ_1C-4 elutes in this position and is approximately 55 residues long.

Amino acid analysis of pool B, Figure 7, shows the presence of hexosamines, indicating that a fragment in this pool derives from the carbohydrate containing section of γ_1 chain, corresponding, at least in part, to γ_2C-3. The elution volume of this pool falls between that of γ_2C-3 and γ_2C-4. In fact these fractions contain a fragment called γ_1C-3 which is approximately 84 residues long and contains the carbohydrate moiety of the γ_1 chain. The presence of radiolabel in this pool may indicate contamination by "C-1-a pieces" (Birshtein et al., 1971) derived from CNBr cleavage of those V_γ regions lacking methionines at either positions N-34 or N-83.

Pool G, Figure 7, contains a fragment which is not homologous to any γ_2 fragment in size or amino acid composition. The fragment appears to be approximately 32 residues in length as judged by its elution after γ_1-C-1-n (34 residues) from the G-50 column and by normalizing its amino acid composition to one homoserine. It is designated γ_1C-2 and may have been disulfide bonded to γ_1C-3 in the parent molecule.

Total reduction and alkylation of the material in pool C (Figure 6) from the G-100 column, and filtration through a column of Sephadex G-50 gave rise to many of the γ_1 CNBr fragments including γ_1C-1-a$_2$, as judged from elution volume, amino acid composition, and content of radiolabel, analogous to γ_2C-1-a$_2$.

Two fragments which account for the remainder of the γ_1 chain are found in pools D, E, and F (Figure 6) from the G-100 column. Fragment γ_1C-1-a$_1$ is the predominant component of pool D, as the elution volume and amino acid composition of pool D correspond to those of γ_2C-1-a$_1$. The carboxyl-terminal fragment, γ_1C-5, is found in pools E and F. Filtration of pool E through a column of Sephadex G-50, as shown in Figure 8, resolves γ_1C-5 (pool B), which lacks homoserine (Table I), from a labeled fragment (pool A). Relevant data from CNBr cleavage of γ_2 chain suggest that this latter fragment may represent the carboxyl-terminal ~35 residues of γ_1C-1-a$_2$. This fragment might derive from a subpopulation of molecules which has a methionine at ~N-105 within the hypervariable section of γ_1C-1-a$_2$ (P. Koo, T. Trischmann, D. Tracey, and J. Cebra, unpublished observations).

Discussion

Nine fragments accounting for the entire ~444 residues of the heavy chain of guinea pig IgG1 have been isolated by gel filtration procedures following CNBr digestion and subsequent reductive cleavage of the disulfide bonds of whole IgG1 and of γ_1 chain. The CNBr fragments isolated either from the γ_1 chain or from whole IgG1 had very similar amino acid compositions, but it is felt that isolation of all fragments from whole IgG1 is the most expedient method, and results in greatest purity of the isolated fragments.

The four fragments from γ_1 chain which have amino acid compositions nearly identical with γ_2 fragments are those homologous to the fragments comprising the amino-terminal half of the γ_2 chain: i.e., γ_2C-1-n, γ_2C-1-a$_1$, γ_2C-1-a$_2$, and γ_2C-1-b. Besides having similar compositions, these γ_1 fragments have elution volumes from Sephadex columns identical with their proposed γ_2 analogues. It has been shown previously that the Fab portions of IgG1 and IgG2 antibodies are quite similar as judged by peptide mapping (Lamm et al., 1967) and amino acid composition (Lamm, 1969; Leslie et al., 1971). Thus, it is likely that fragments γ_1C-1-n, γ_1C-1-a$_1$, γ_1C-1-a$_2$, and γ_1C-1-b together comprise most of the amino-terminal half of the γ_1 chain and are aligned in an order analogous to the γ_2 fragments (Benjamin et al., 1972).

The fragment which has been designated γ_1C-1-c most likely originates from the central or "hinge" region of the γ_1 chain. It is the same length as γ_2C-1-c and is rich in prolyl and half-cystinyl residues, analogous to γ_2C-1-c. In addition, this fragment bears considerable radiolabel after mild reduction and radioalkylation of IgG1, consistent with the analogous finding with γ_2C-1-c from IgG2 (Birshtein et al., 1971).

The only other γ_1 chain fragment obviously analogous to a γ_2-chain fragment is the carboxyl-terminal γ_1C-5, identified as such because of its lack of homoserine. The amino acid composition of γ_1C-5 correlates well with the published composition and sequence of the carboxyl-terminal portion of an IgG1 peptic fragment (Melamed, 1973).

The remaining γ_1-chain fragments γ_1C-2, γ_1C-3, and

γ_1C-4 have no γ_2 analogues. Together these fragments account for ~180 residues and probably originate from the Fc region of the γ_1 chain. Fragment γ_1C-3 was the only γ_1 fragment bearing sugar residues and these are probably located near residue N-293 in the Fc region where an oligosaccharide is attached to the γ_2 chain (Tracey and Cebra, 1974).

Amino acid composition data (Leslie and Cohen, 1970) of H chains suggest that the γ_1 chain has one more methionyl residue than the γ_2 chain. This finding is supported by our isolation of nine CNBr fragments from γ_1 chain, whereas eight fragments account for the γ_2 chain. Thus it is likely that our nine fragments do account for the entirety of the γ_1 chain, as the γ_2 fragments have been rigorously aligned and shown to account for the whole heavy chain (Benjamin et al., 1972).

The probable alignment of the γ_1 chain CNBr fragments and the arrangement of disulfide bonds within the IgG1 molecule have almost been completed (S. Graziano, S. Liu, D. Tracey, unpublished data). With the framework of the IgG1 molecule thus set out, we have begun direct comparisons of the V regions of IgG1 and IgG2 antibodies of the same ligand binding specificity using selective modification by affinity labels and primary structural analysis (T. Trischmann, M. Ricardo, S. Liu, and D. Tracey, unpublished observations).

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On the Analysis of Circular Dichroic Spectra of Proteins†

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ABSTRACT: A new method is presented for analyzing circular dichroism spectra. The method employs integrals over the data and calculates the α-helical, β-sheet, and random coil content of the proteins from such integrals. It is shown that the analyzed α-helical content is usually reliable to within 5%, β-sheet values are somewhat less reliable, and random coil values are least reliable. Curve fitting techniques are shown to be misleading. The method has a number of advantages over existing procedures.

Circular dichroism (CD) is widely used to obtain information on the secondary structure of proteins and nucleic acids in solution. The technique is sensitive, and only dilute solutions are needed. The raw CD data themselves, however, are not of direct concern to an investigator. What is of interest is the content of various types of secondary structure.

It has become customary to divide CD spectra into three types—α helical, β sheet, and random coil. Random coil is a miscellaneous category which bears no necessary relationship to a hydrodynamic random coil, nor does it necessarily

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