The exclusion of $c \rightarrow 0$ is not a serious limitation, since in order for the experiments to be meaningful, the concentration of added buffer must be substantially higher than that of the endogenous buffer groups.

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Primary Structure of C-1-a_1—a Cyanogen Bromide Fragment of Heavy Chain from Inbred Guinea Pig Immunoglobulin G(2), Which Contains a Markedly Variable Segment*

Barbara K. Birshtein† and John J. Cebra‡

ABSTRACT: The primary structure of a 49-residue CNBr fragment from the N-terminal quarter of strain 13 guinea pig immunoglobulin G(2) heavy chain has been determined. This fragment, which spans residues N-35 to N-83 of the γ_2 chain, has two regions of almost constant sequence joined by a markedly variable region spanning 12 residues. Automated sequential degradation of C-1-a_1 from strain 13 and strain 2 animals and from anti-dinitrophenyl antibody purified from strain 13 animals confirmed the presence of a constant sequence for residues N-36 to N-47 of γ_2 chain. There was greatly restricted variability in purified antibody within the region shown to be markedly variable in normal C-1-a_1 of both strains. Positions N-49, N-51, and N-58 within this variable region seem constant in all these sources of C-1-a_1.

We have presented the procedures for the isolation of CNBr fragments accounting for 303 residues from the C-terminal three-quarters of the γ_2[1] chain from strain 13 inbred guinea pig IgG(2)[2] (Birshtein *et al.*, 1971a). Sequence data of two of these fragments and of a tryptic peptide containing methionine have shown a constant sequence for that segment of the molecule extending from position ~N-120 to the hinge region in the middle of the chain (Turner and Cebra, 1971; Birshtein *et al.*, 1971b). Position N-120 is very close to that residue position which, in myeloma proteins, is termed the "switch point," that point N terminal to which these proteins have differing sequences.

The isolation of two additional fragments (C-1-a_1 and C-1-a_2) was reported (Birshtein *et al.*, 1971a). These fragments as well as a third, C-1-n, have been shown to account for the

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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.

[2] Abbreviations used are: IgG(2), immunoglobulin G(2); DNP and TNP, di- and trinitrophenyl; HSA, human serum albumin; PTH, phenylthiohydantoin; PCA, pyrrolidonecarboxylic acid.

N-terminal 140 residues of γ_2 chain (D. C. Benjamin and Q. Z. Hussain, unpublished data). A procedure is presented here which permits isolation of these three CNBr fragments, C-1-n, C-1-a_1, and C-1-a_2, directly from intact IgG(2) after only one or two fractionation steps. These fragments together correspond to V_H, the variable region of heavy chain from myeloma proteins. Since these fragments appear to be implicated in forming the antigen binding site (Ray and Cebra, 1971), determination of their basic sequences in normal IgG(2) and specific antibodies should facilitate the correlation of primary structure with specificity for antigens.

The amino acid sequence of C-1-a_1, which spans residues N-35 to N-83, is discussed in this paper. The pattern of variability within C-1-a_1 was established with the aid of various enzymatic digests and by automated sequential Edman degradation. The ability to isolate this fragment easily and reproducibly has enabled us to compare the pattern of variability in this segment of strain 13 γ_2 chain with the equivalent region from strain 2 animals and from purified anti-dinitrophenyl antibody raised in strain 13 animals.

Materials and Methods

Animals. Strain 13 and strain 2 inbred guinea pigs were used. Strain 2 animals were a gift of Dr. Ira Green of NIAID, Bethesda, Md., while strain 13 animals were from our own colony.

Preparation of Hapten–Protein Conjugates. Dinitrophenyl (DNP) groups were attached to limpet hemocyanin (Mann) by reacting it with dinitrobenzenesulfonic acid, sodium salt (Eastman) (Eisen, 1964). The ratio of optical densities of this antigen at 360 mμ:280 mμ was 1.4 at pH 7.4.

Trinitrophenyl (TNP) groups were conjugated to human serum albumin (HSA) (Pentex fraction V) with the use of trinitrobenzenesulfonic acid (K & K Laboratories) (Little and Eisen, 1966). The ratio of optical densities of this antigen at 348 mμ:280 mμ was 2.6 at pH 7.4.

Dinitrophenyl groups were attached to HSA with the use of fluorodinitrobenzene (Matheson). The conjugate had 62 moles of DNP/mole of HSA.

Preparation of Anti-DNP Antibody: Immunization. Strain 13 guinea pigs were immunized in the four footpads with a total of 1 mg of DNP-hemocyanin emulsified in complete Freund's adjuvant. Three weeks later, guinea pigs were skin tested with DNP-hemocyanin in saline three times at 1-day intervals. Each time a total of 50 μg of DNP-hemocyanin was injected intradermally into four sites. Serum was collected by cardiac puncture of the animals during the 3 weeks following skin testing. Anti-DNP antibody elicited by this treatment is predominantly IgG(2) (Asherson and Stone, 1965).

Isolation of Anti-dinitrophenyl Antibody from Strain 13 Guinea Pigs. Two methods were used. (1) The immunoglobulin fraction of serum from immunized guinea pigs was precipitated at 37% saturation with ammonium sulfate, washed three times with 37% saturated ammonium sulfate, and dissolved in phosphate-buffered saline. Specific anti-hemocyanin antibody was removed by precipitation with hemocyanin, and then specific anti-DNP antibody was prepared by precipitation with TNP-HSA and subsequent dissociation and isolation, according to the method of Little and Eisen (1966). (2) Specific anti-DNP antibody was precipitated from the serum of immunized guinea pigs by stepwise addition of DNP-HSA. The antibody–antigen precipitate was collected by centrifugation and was solubilized by addition of ϵ-DNP-lysine. This solution was passed through a column of DEAE-cellulose, equilibrated in 0.15 M sodium phosphate (pH 7.5), 0.3 M in NaCl, which retained DNP-HSA. The mixture of antibody and ϵ-DNP-lysine was eluted directly with this buffer. Dialysis of the mixture against the same buffer removed unbound ϵ-DNP-lysine. The antibody solution was then dialyzed against distilled water and freeze-dried.

Preparation of IgG(2). Animals were immunized with a variety of hapten–protein conjugates. With one exception, in which anti-DNP antibody was not isolated from the immune serum, specific antibody was removed from either the serum or from that fraction of the serum precipitated at 37% saturation with ammonium sulfate by stepwise precipitation with hapten coupled to a heterologous carrier. Immunoglobulin G(2) was isolated by passage of the serum, or that fraction of the serum separated by ammonium sulfate precipitation, through a column of DEAE-cellulose, equilibrated in urea–phosphate buffer, as described previously (Birshtein *et al.*, 1971a).

Preparation of C-1-a_1 from CNBr Cleavage of γ_2 Chain. Fragment C-1-a_1 was isolated as described previously (Birshtein *et al.*, 1971a). C-1-a_1, isolated in this way, was used for all determinations of primary structure except where indicated otherwise. In outline, heavy chain from IgG(2) and from anti-DNP antibody prepared by the method of Little and Eisen (1966) was isolated after mild reduction and alkylation of the interchain disulfide bonds. The γ_2 chain was subjected to CNBr cleavage and the reaction mixture was resolved by gel filtration on a column of Sephadex G-100, equilibrated in 8 M urea, 0.1 M in formic acid. Pool CB4, whose major component was C-1-a_1, was often used without further purification. However, when separation of pool CB4 from CB3 seemed inadequate, C-1-a_1 was isolated after further recycling of pool CB4 on a column of Sephadex G-50, equilibrated in 8 M urea, 0.1 M in formic acid.

Isolation of C-1-a_1 and Other Fragments Comprising γ_2 Chain after CNBr Cleavage of IgG(2). The freeze-dried anti-DNP antibody purified by precipitation with DNP-HSA, solubilization of the precipitate with ϵ-DNP-lysine, and subsequent ion-exchange chromatography was subjected to CNBr cleavage (Birshtein *et al.*, 1971a) without initial separation of heavy and light chains. The reaction was stopped by the addition of water, and the reaction mixture was immediately freeze-dried. Freeze-dried antibody ($\sim$200 mg), after CNBr cleavage, was allowed to stand for 12 hr at room temperature in 8 ml of 8 M urea, 0.1 M in formic acid. The solution was then applied to a column of Sephadex G-100 (3.8 $\times$ 140 cm), equilibrated in the same solvent. The elution profile is shown in Figure 1. Pools were freed of urea by gel filtration, as described earlier (Birshtein *et al.*, 1971a).

Fragment C-1-a_1 was the predominant component of pool CBVI and was used without further purification. Pool CBI probably consisted of aggregated material. After total reduction and alkylation (Birshtein *et al.*, 1971a) and subsequent gel filtration, pool CBII yielded fragments C-1-n and C-1-a_2 as well as light chain, and pool CBIII gave fragments C-1-c and C-1-b. Pools CBIV, V, and VII contained fragments C-3, C-4, and C-5, respectively. Isolation of these fragments, which account for the entire γ_2 chain, can thus be achieved without initial separation of heavy and light chains.

Enzymatic Digestion of C-1-a_1 from Strain 13 Guinea Pig γ_2 Chain. This fragment (0.7–2.0 μmoles) was dissolved in 5 ml of 0.05 M NH_4OH, and the pH was adjusted to 8.0 with 0.05 M acetic acid. Trypsin, chymotrypsin, or thermolysin was added (two portions of 100–150 μg each), and the digestion mixture was incubated at 37° for 4–6 hr. With the exception

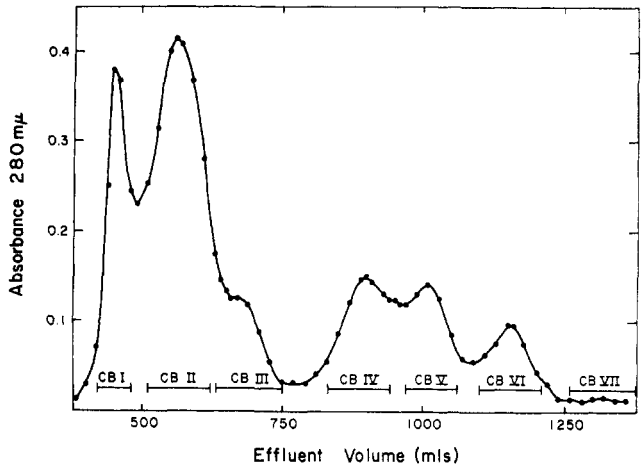

FIGURE 1: Fractionation of CNBr fragments from strain 13 anti-DNP antibody on a column of Sephadex G-100 (3.8 × 140 cm), equilibrated in 8 M urea, 0.1 M in formic acid. Details of the procedure are found in the text. Fraction size was 10 ml.

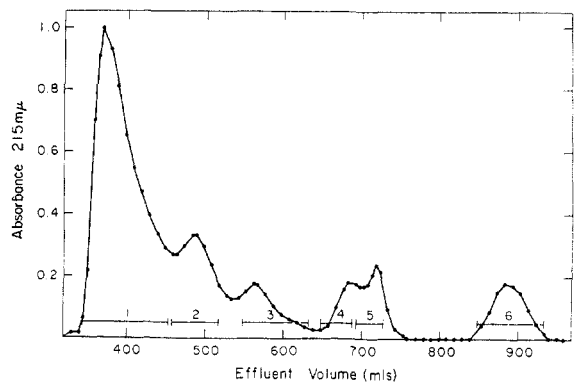

FIGURE 3: Elution profile of the separation of the peptides resulting from a tryptic digest of C-1-a_1 on a column of Sephadex G-25, fine (2.0 × 240 cm), equilibrated in 0.05 M NH_4OH. Details of the procedure are found in the text. Fraction size was 5 ml.

of the chymotryptic digestion, all digests were done on C-1-a_1 from IgG(2) devoid of specific anti-hapten antibody. Chymotryptic digestion was done on C-1-a_1 from IgG(2) containing specific anti-DNP antibody.

Automated Sequential Degradation. Separate preparations of C-1-a_1 were automatically and sequentially degraded in the Beckman Model 890 sequencer. These preparations were: (1) 1.0 μmole of C-1-a_1 from strain 2 animals; (2) 1.0 μmole of C-1-a_1 from strain 13 animals (both of these were free of specific anti-hapten antibody); (3) 0.56 μmole of C-1-a_1 from anti-DNP antibody, purified by the method of Little and Eisen; and (4) 0.77 μmole of C-1-a_1 from a 1:1 mixture of anti-DNP antibody from the same source as 3 and from anti-DNP antibody prepared by precipitation with DNP-HSA and CNBr cleavage of the intact molecule.

The resulting thiazolinone derivatives of the amino acid residues were converted to the corresponding phenylthiohydantoins (PTH's) by incubation in 0.2 ml of 1 N HCl at 80° for 10 min. The PTH-amino acid derivatives were extracted into ethyl acetate and were then identified by gas chromatography on a Beckman GC-45 gas chromatograph (Pisano and Bronzert, 1969), by thin-layer chromatography on silica gel sheets (Eastman) (Jeppsson and Sjöquist, 1967), or by regeneration of the amino acid by acid hydrolysis for 24 hr at 150° (Van Orden and Carpenter, 1964) in an oil bath equipped with a condenser, followed by analysis on a Beckman amino acid analyzer, Model 120B or 120C. In our hands, serine was seldom obtained and identified, either as its PTH derivative or as the free amino acid.

The amino-terminal pyrrolidonecarboxylic acid which occurred in peptide T49 was removed by treatment with pyrrolidonecarboxylic acid hydrolase (PCA-hydrolase), as described (Turner and Cebra, 1971).

The nomenclature of tryptic peptides follows the rules established earlier (Turner and Cebra, 1971), in that peptides are numbered consecutively from the C terminus. T37–T42 have been reserved for peptides from CNBr fragment C-1-a_2, although only peptides T37, T41, and T42 have thus far been isolated and their sequences determined. Consequently, C-1-a_1 is composed of tryptic peptides T43–T50. Fragment C-1-a_1 spans residue positions N-35 to N-83 in γ_2 chain. All residue positions discussed in the text will refer to the parent γ_2 chain.

Other Methods. Ion-exchange chromatography of tryptic and chymotryptic digests on columns of Dowex 1-X2 was done as described previously (Birshtein *et al.*, 1971b). Enzyme digests were resolved by gel filtration on columns of Sephadex G-25, fine (2 × 240 cm), and Sephadex G-50, fine (1.75 × 186 cm), both equilibrated in 0.05 M NH_4OH.

High-voltage electrophoresis of peptides at pH 3.6 and 6.5 and amino acid sequence analysis of component peptides were carried out as previously described (Turner and Cebra, 1971). Whether Asx or Glx in peptides was present as the acid or amide was deduced from the mobility of the peptide at pH 6.5.

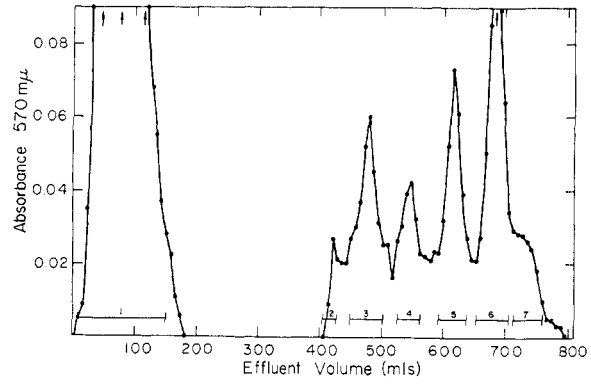

FIGURE 2: Separation of the tryptic peptides of C-1-a_1 on a column of Dowex 1-X2. Details of the procedure are found in the text. The column was developed at 1 ml/min with a continuous gradient of pyridinium acetate buffers. A portion of the effluent (0.14 ml/min) was automatically analyzed by the ninhydrin reaction after alkaline hydrolysis. The absorbance of the reaction mixture was monitored at 570 mμ.

Results

Isolation of Tryptic, Chymotryptic, and Thermolytic Peptides from C-1-a_1. The amino acid compositions of C-1-a_1 from strain 2 and strain 13 animals are shown in Table I. Tryptic peptides of strain 13 C-1-a_1, isolated from IgG(2) depleted of specific anti-DNP antibodies, were initially fractionated either by ion-exchange chromatography on Dowex 1-X2, as shown in Figure 2, or, more simply, by gel filtration, giving the elution profile shown in Figure 3. Subsequent application of gel filtration and preparative high-voltage electrophoresis resulted in the isolation of these peptides, as outlined in

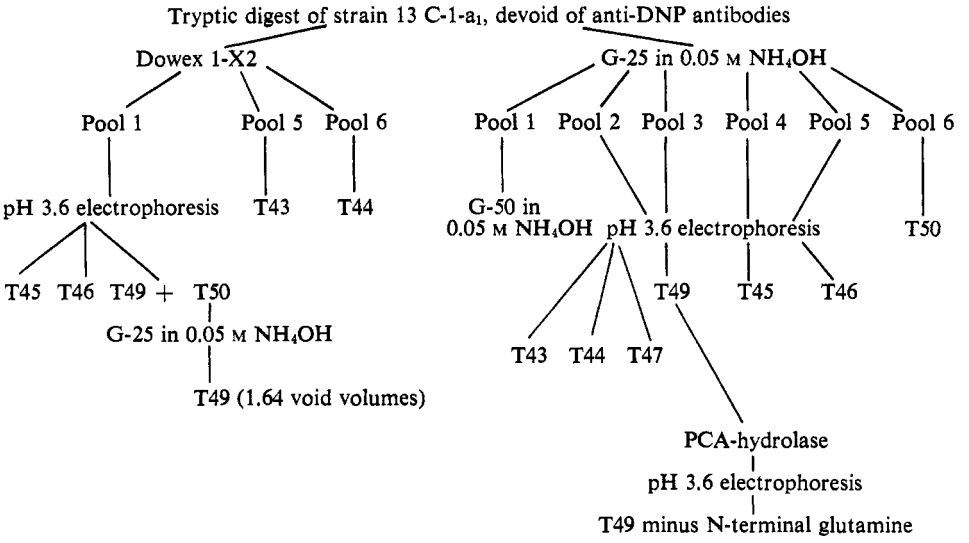

FIGURE 4: An outline of the steps used to isolate the tryptic peptides of C-1-a1.

Figure 4. The amino acid compositions of these peptides and their relative mobilities are found in Table I. Tryptic peptide T47 was isolated in low yield compared to the other tryptic peptides.

Strain 13 C-1-a1, isolated from IgG(2) containing anti-DNP antibodies, was used for chymotryptic cleavage. Peptides were initially resolved by ion-exchange chromatography, as shown in Figure 5. Chymotryptic peptides used for structural studies were isolated by the methods shown in Figure 6.

Strain 13 C-1-a1, from IgG(2) depleted of anti-DNP antibodies, was subjected to thermolytic cleavage. Initial fractionation of the digest was done by gel filtration, and the elution profile is shown in Figure 7. Thermolytic peptide Th3J was isolated according to the methods outlined in Figure 8.

Analyses of these chymotryptic and thermolytic peptides and their relative mobilities can be found in Table I.

The amino acid sequence of these peptides is shown in Figure 9.

Automated Sequential Degradation of C-1-a1. Automated sequential degradation of C-1-a1 yielded the results shown in Figure 9.

Segments Having a Single Amino Acid Sequence in C-1-a1. Figure 9 shows that C-1-a1 contains segments at the N and C termini having a single amino acid sequence. The sequence at the N terminus, from N-36 to N-47, was established by the automated sequential degradation of C-1-a1 and by the isolation of tryptic peptides T50 and T49. Heterogeneity at the N-terminal position is indicated since some alanine was identified at this position during the sequencer determination, while serine was shown to be the N-terminal residue of peptide T50. Peptide T49 was treated with the enzyme PCA-hydrolase to remove the cyclized glutamine residue before further sequence determination.

The single sequence at the C terminus, from N-60 to N-83, was determined by the isolation of overlapping component peptides and automated sequential degradation, as indicated in Figure 9.

Variability within C-1-a1. Automated sequential degradation of C-1-a1 from strain 13 and strain 2 IgG(2), devoid of specific anti-hapten antibody (referred to as "normal"), clearly indicated the presence of a markedly variable region spanning residue positions N-48 to N-59, as shown in Figure 9. Alternative residues at the same positions within this variable region are generally, although not entirely, the same for the two strains. Position 49 seems to be occupied by a constant threonine and positions 51 and 58 by constant isoleucines.

Automated sequential degradation of C-1-a1, derived from

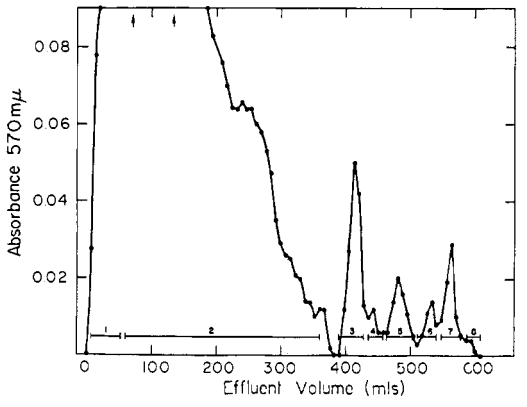

FIGURE 5: Separation of the chymotryptic digest of C-1-a1 on a column of Dowex 1-X2. Details of the procedure are found in the text and in the legend to Figure 2.

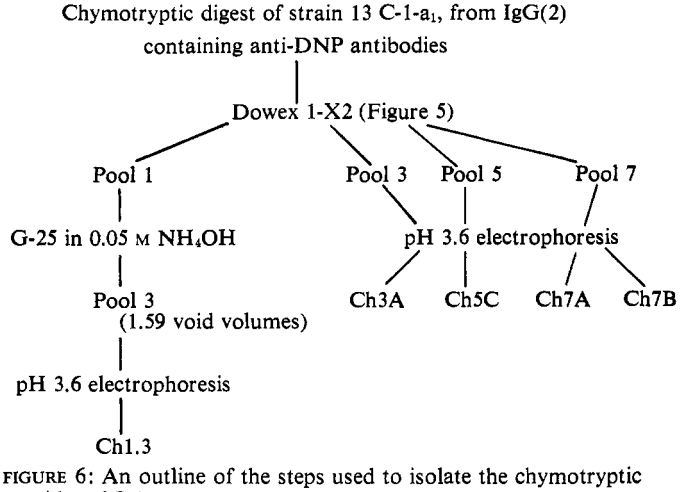

FIGURE 6: An outline of the steps used to isolate the chymotryptic peptides of C-1-a1.

TABLE I: Fragment C-1-a_1 and Tryptic, Chymotryptic, and Thermolytic Peptides Derived from It.[a]

	Strain 13 C-1-a_1	Strain 2 C-1-a_1	Strain 13 Tryptic Peptides							Strain 13 Chymotryptic Peptides					Strain 13 Thermolytic Peptide
			T43	T44	T45	T46	T47	T49	T50	Ch1.3	Ch3A	Ch5C	Ch7A	Ch7B	Th3J
Lys	2.9	2.6		0.81			0.87	0.98		1.0					0.79
His	0.35	0.51													
Arg	2.8	2.6			0.90	0.80			0.91	0.78					0.74
Asp	4.6	4.7	1.0	2.0			1.2			1.0		1.0			2.8
Thr	3.4	3.8	0.91		1.0							0.98	0.90	1.1	1.0
Ser	4.1	4.8	0.50	0.61	1.0		1.6		1.2	1.0		1.0	0.62		1.1
Glu	3.1	3.0	1.0					1.0			0.95				
Pro	1.8	2.2						0.80							
Gly	5.0	4.8	0.41	1.1		1.2	1.0	1.1		1.3		3.6			1.1
Ala	2.6	3.1	0.14				1.1	1.0		1.0					
Val	2.6	3.1	0.25				1.0			0.96				0.89	
Ile	2.8	2.5			1.1				0.86			1.7			0.80
Leu	3.4	3.3	1.7								1.0		1.0		
Tyr	2.3	2.2	0.91				0.71					0.72	1.1		
Phe	1.4	1.5			0.90					0.90					
Trp	+	+							+					+	
Hsr	1.2	1.0	0.83								1.0				
Mobility[b]															
pH 3.6			+0.06		+0.59	+0.94	+0.45	+0.67	+0.62	+0.63	+0.24	+0.09	+0.22	+0.28	+0.50
pH 6.5				−0.51											

[a] Compositions are reported as moles of amino acid per mole of peptide. [b] Mobility given relative to lysine = +1.0, aspartic acid = −1.0, neutral amino acid = 0.

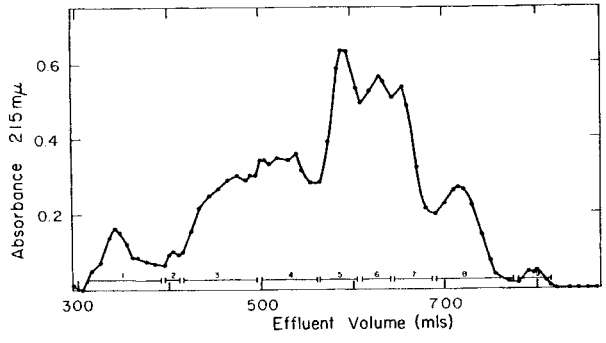

FIGURE 7: Elution profile of the separation of the peptides resulting from a thermolytic digest of C-1-a_1 on a column of Sephadex G-25, fine (2.0 × 240 cm), equilibrated in 0.05 M NH_4OH. Details of the procedure are found in the text. Fraction size was 5 ml. The absorbance was monitored at 215 mμ.

Thermolytic digest of strain 13 C-1-a_1 from IgG(2), depleted of anti-DNP antibodies

↓

G-25 in 0.05 M NH_4OH (Figure 7)

↓

Pool 3

↓

pH 3.6 electrophoresis

↓

Th3J

FIGURE 8: An outline of the steps used to isolate the thermolytic peptide Th3J of C-1-a_1.

strain 13 anti-DNP antibody, showed the sequence of the N-terminal 12 residues to be identical with that found in the normal C-1-a_1. No residue could be identified in position 47 from anti-DNP antibody. However, the identification of the presence of tryptophan at this position may well be obscured by a lowered yield of its PTH derivative since sequence determination of anti-DNP antibody was carried out on about two-thirds the material used for other sequencer determinations.

Position N-48 in sequence analysis of normal C-1-a_1 heralds the beginning of a markedly variable region. Alternatives at position 48 include Ile, Leu, and Val in approximately a 2:1:1 ratio. In contrast, position 48 in anti-DNP antibodies has valine almost exclusively. These results are tabulated in Table II. Positions 49 and 50 in anti-DNP antibodies also seem to have single residues, Thr and Trp, respectively, while position 50 in normal C-1-a_1 is occupied by several alternatives which include Trp. Positions 51 and 58 probably have constant isoleucine in both populations.

An attempt to isolate tryptic peptide(s) T48 from the region including positions 44–59 was made by fractionating a digest of C-1-a_1 by gel chromatography on Sephadex G-25, as shown in Figure 3. Since the six known and tentatively positioned tryptic peptides T43 to T46 and T49 and T50 were isolated

TABLE II: Position N-48 in C-1-a_1 Derived from Strain 2 and Strain 13 γ_2 and from Strain 13 Anti-DNP Antibody—Alternative Residues Present and Relative Yields.[a]

	Normal C-1-a_1				Anti-DNP C-1-a_1[c]		
	Strain 2		Strain 13		Strain 13		
	GC[b] (%)	Regeneration (%)	GC (%)	Regeneration (%)	GC (%)	Regeneration (%)	GC (%)
Ile		53		51		8	
	73		80		5		6
Leu		29		20		3	
Val	27	18	20	29	95	89	94

[a] Only Ile, Leu, and Val were seen as alternative residues at position 48 in these determinations. Consequently, relative percentages were calculated on the basis of Ile + Leu + Val = 100%. [b] In our hands, Ile and Leu cannot be distinguished by gas chromatography (GC). The peak height determined for an isoleucine standard was used in these calculations. [c] The first two columns represent data of GC and amino acid regeneration from a single run. The GC data in the third column are from a second sequencer analysis of a different sample.

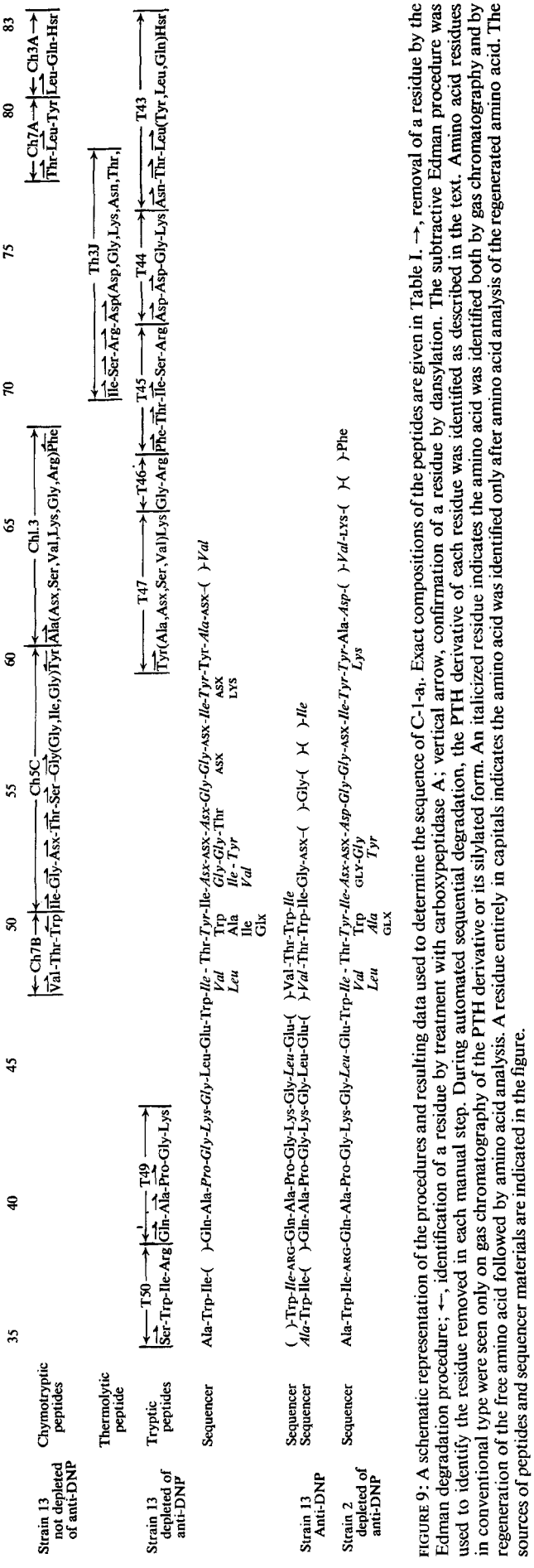

FIGURE 9: A schematic representation of the procedures and resulting data used to determine the sequence of C-1-a_1. Exact compositions of the peptides are given in Table I. →, removal of a residue by the Edman degradation procedure; ←, identification of a residue by treatment with carboxypeptidase A; vertical arrow, confirmation of a residue by dansylation. The subtractive Edman procedure was used to identify the residue removed in each manual step. During automated sequential degradation, the PTH derivative of each residue was identified as described in the text. Amino acid residues in conventional type were seen only on gas chromatography of the PTH derivative or its silylated form. An italicized residue indicates the amino acid was identified both by gas chromatography and by regeneration of the free amino acid followed by amino acid analysis. A residue entirely in capitals indicates the amino acid was identified only after amino acid analysis of the regenerated amino acid. The sources of peptides and sequencer materials are indicated in the figure.

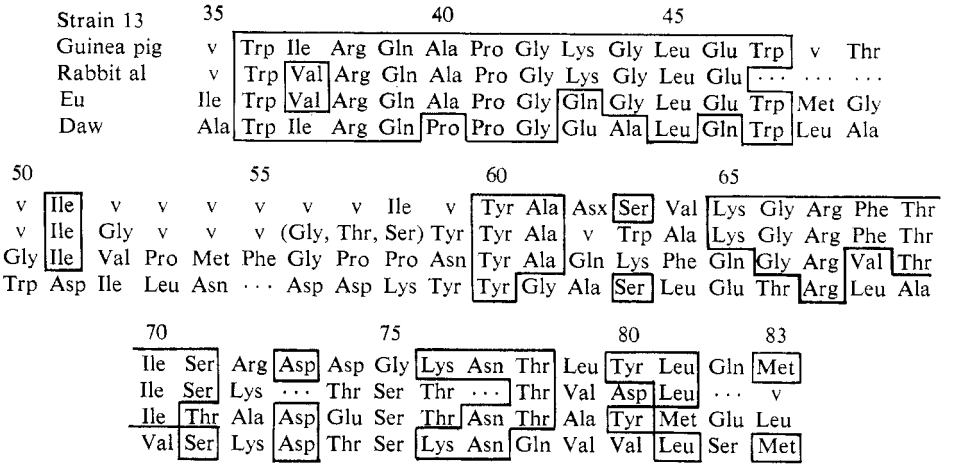

FIGURE 10: The sequence of C-1-a_1 from guinea pig γ_2 chain compared with corresponding sequences from rabbit γ chain (allotype a1), myeloma heavy chain from Eu of subgroup V_{HI}, and myeloma heavy chain from Daw of subgroup V_{HII}. Those residues identical at a given position with the one found in guinea pig C-1-a_2 are included in closed rectangles. Residue positions in the sequence for guinea pig and rabbit where alternative amino acids have been found are indicated by "v" for "variable." Sequence data of rabbit γ chain are from Mole *et al.* (1971), for Eu protein from Edelman *et al.* (1969), and for Daw protein from Press and Hogg (1969). Deletions or "gaps" have been introduced into the sequence for rabbit γ_2 chain to allow maximum correspondence of it with that of guinea pig C-1-a_1. These gaps, indicated by (- - -), are not necessarily between residue positions separated by Mole *et al.* (1971) when they compared their data with other sequences.

from pools 2 to 6 and since pool 2 contained tryptic peptide T47 as well, it was thought that pool 1 must contain tryptic peptides accounting for the intervening region. However, a subsequent gel filtration step of pool 1 of Figure 3 on Sephadex G-50 did not resolve peptides sufficiently to allow their characterization.

A chymotryptic digest of C-1-a_1, from strain 13 IgG(2) containing specific anti-DNP antibodies, yielded two peptides tentatively positioned in the variable segment of C-1-a_1. These are Ch5C and Ch7B. They have been placed as shown in Figure 9. Thus, it is possible that there is a simpler sequence for C-1-a_1 from purified antibodies in the section found to be variable in normal C-1-a_1, and that one can isolate peptides accounting for this section from enzymatic digests of C-1-a_1 from specific antibodies or from IgG(2) containing specific antibodies.

Discussion

Residue positions N-36 to 47 and 60 to 83 are relatively constant in the variety of C-1-a_1's that have been studied, either by automated sequential degradation from the N terminus or by analysis of various enzymatic digests. By contrast, the heterogeneity in residue positions 48 to 59 is striking, although specific positions 49, 51, and 58 may be constant. Since purified anti-DNP antibody showed more restricted heterogeneity than did "normal" C-1-a_1, variability in the middle of C-1-a_1 could correlate with antibody specificity.

The fragment C-1-a_1 accounts for residue positions N-35 to N-83 of γ_2 chain from guinea pig IgG(2) (D. C. Benjamin and Q. Z. Hussain, unpublished data). The corresponding sections of heavy chains from human myeloma proteins occur in their "variable" region (V_H), that part where different myeloma proteins of the same Ig class have different amino acid sequences. Recently the sequences of the first 110 residues of both heavy and light chains of myeloma proteins have been ordered into subgroups (Hood and Talmage, 1970; Köhler *et al.*, 1970; Capra, 1971). Myeloma heavy chains of a particular subgroup have similar sequences in the section corresponding to C-1-a_1 except in that region, positions 48–59, where the fragment from "normal" guinea pig γ_2 chain shows great variability. Thus, although some of the variable residue positions in C-1-a_1 might correlate with the expression of a particular subgroup in normal guinea pig immunoglobulins, the close correspondence between the "variable" section of C-1-a_1 and one of the "hypervariable" sections of myeloma heavy chains of a particular subgroup suggests that these positions may be involved in specifying antigen binding activity.

A comparison of the primary structure of guinea pig C-1-a_1 with the corresponding amino acid sequences from Daw (Press and Hogg, 1969) and Eu (Edelman *et al.*, 1969), the first two myeloma proteins examined in this detail, is given in Figure 10. Judging by only the Eu (V_{HI}) and Daw (V_{HIII}) proteins, one would have expected to encounter alternative residues not only at residue positions 48–59 of C-1-a_1 but also in the relatively constant section from positions 60 to 83. Perhaps the guinea pig does not display heterogeneity of immunoglobulins with respect to heavy chain subgroups or perhaps one subgroup is naturally or artificially dominant in our preparations. Amino acid sequence data for the N-terminal 98 residues of guinea pig γ_2 chain (D. C. Benjamin and A. Ray, unpublished data), including C-1-a_1, indicate that it corresponds most closely with subgroup V_{HIII} of human myeloma proteins (J. D. Capra, personal communication). The source materials used for these sequencer studies were derived from serum obtained after different immunization schedules and, in part, from animals injected with different antigens. Whether an antigen can divert a significant fraction of total immunoglobulin production into the making of a normally minor subgroup, even though only a fraction of antibody molecules produced is specific, is not known; however, the amount of IgG(2) per milliliter of serum which can be isolated from immunized animals, even when specific anti-hapten antibody has been removed, is approximately double that from normal animals (Birshtein *et al.*, 1971a). It is therefore not unlikely that a deflection of this type could occur.

Alternative residues in the N- and C-terminal segments of

C-1-a_1 have not been confirmed, with the exception of a Ser–Ala interchange at position 35. It is known, however, that residues 35–83 are included in larger CNBr fragments found in pool CB2-I (Birshtein *et al.*, 1971a). Perhaps variation in these segments, consonant with the presence of subgroups, may come to be defined.

Data concerning the primary structure of "normal" rabbit IgG has been made available to us and will shortly be published (Mole *et al.*, 1971). The amino acid sequence from the section of rabbit heavy chain corresponding to C-1-a_1 is also given in Figure 10. In this species too there seems to be a relatively constant amino acid sequence accounting for much of the region corresponding to V_H of myeloma proteins. Of the nine residue positions between N-48 and N-59 that show alternative amino acids in guinea pig C-1-a_1, four can be made to correspond to variable positions in the rabbit sequence (Figure 10).

Genetic polymorphism in a group of outbred animals might be expected to be reflected by amino acid variability at certain positions in immunoglobulin heavy chains were a pool of serum from many individuals used to prepare IgG for analysis. Our own C-1-a_1, from strain 13 inbred guinea pigs, would not be expected to be a mixture of allotypes. Thus we feel that the best explanation for the variability of positions 48–59 in C-1-a_1 from "normal" γ_2 chain and the considerable restriction on alternative residues at some of these positions in anti-DNP antibody is that this "variable" section is involved in determining antigen binding specificity.

The size of C-1-a_1 and its pattern of variability—constancy at the amino and carboxyl termini—make it amenable to automated sequential degradation. Comparison of C-1-a_1 from various sources is thus facilitated. Inspection of C-1-a_1 from purified antibodies of a variety of specificities should indicate whether the sequence of its variable section correlates with antibody activity and hence may be involved in forming the antigen binding site.

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