Primary Structure of the “Hinge” Region of Human IgG3

PROBABLE QUADRUPLETION OF A 15-AMINO ACID RESIDUE BASIC UNIT*

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The middle part of the heavy chain of IgG3 (hinge region) which covalently links the two γ3 chains to each other, is about 4 times larger than the same region in the three other human IgG subclasses. This is probably due to a quadrupletion of a 45-nucleotide DNA segment resulting in a γ3 hinge region which is 62 amino acid residues long and consists of an NH2-terminal 17-residue segment followed by a 15-residue segment which is identically and consecutively repeated three times. The NH2-terminal 17-residue segment shows 70% homology with the repetitive 15-residue segment and appears to be the result of a small insertion and several point mutations of the same 45-nucleotide DNA stretch. Since this unit of repetition shows 60 to 70% homology with the hinge of the other IgG subclasses, it may represent the primitive IgG hinge.

It has recently become evident that human IgG3 has structural and biological features not shared by the other three subclasses of human IgG. Functionally, IgG3 does not bind to staphylococcal protein A as do the other subclasses (1), has a more rapid turnover (2), and is the most potent subclass in agglutination-inhibition techniques (3). Structurally, the γ3 chain has a larger molecular weight than other human γ chains (4–6) and IgG3 molecules have an unusually large radius of gyration in aqueous solutions (6, 7). Both features are a result of an unusually large middle part (hinge region) present in the γ3 chain (6, 7). Furthermore, this region of γ3 has a strikingly well ordered secondary and tertiary structure revealed by very simple circular dichroism spectra of an isolated hinge fragment, ctFh (8). The IgG3 subclass is also characterized by a great genetic polymorphism (9). Previous studies of γ3 heavy chain disease proteins have shown that these unusual features are the result of a series of duplications involving the cysteine residues of the hinge (10, 11). The relationship of the extended hinge region to the biological features of IgG3 is still an open question. The aim of the present report is to determine the complete primary structure of the hinge region of normal sized γ3 chains and to define precisely the number of cysteine residues, the order of the repeated sequences, and the relationship of the hinge region to the Cy1 and Cy2 domains.

MATERIALS AND METHODS

Myeloma Proteins (IgG3)–IgG3 proteins were isolated from the sera of three patients (HER, FRO, and JON) with multiple myelomas as previously described (6) using DEAE-cellulose (Whatman DE52) or quaternary aminomethyl (QAE)-Sephadex. For some experiments, a fourth protein, a cryoglobulin (KUP) was used which was isolated by repeated precipitation at low temperature (12). The allotypes of the four IgG3 preparations were determined by agglutination-inhibition techniques (9) to be HER Gm(α), JON Gm(α), FRO Gm(β), and KUP Gm(β).

Gel Filtration—Columns packed with different kinds of Sephadex were used and operated under neutral or dissociating conditions as previously described (b). Standard curves showing the relationship between k, (13) and molecular weights were constructed for each of the columns employing standard proteins (6).

Isoelectric Focusing—Isoelectric focusing was performed using a LKB (Bromma, Sweden) 8101 (110 ml) column, focusing in 1% Ampholine (LKB) at pH 5 to 7.

Isolation of Chymotryptic Hinge Fragment (ctFh)–The ctFh fragment was isolated from three of the IgG3s essentially as described before (14). Briefly, the IgG3 proteins were digested with α-chymotrypsin (Sigma, crystallized three times) (1/50, w/w, enzyme/substrate ratio) in 0.1 M phosphate buffer, pH 7, for 4 h at 37°C. The digest was dialyzed and subjected to gel filtration on a column of Sephadex G-100 in 5 M guanidine HCl, 1 M acetic acid (Fig. 1a). The main optical density peak contained monovalent ctFab (M, = 44,000) and was well separated from the ctFh -containing peak which was detected by an anti-Fh specific antiserum (14). The ctFh fragments were further purified (after dialysis against distilled water) by isoelectric focusing (Fig. 1b).

Isolation of Papain Hinge Fragment (pFh)–IgG3 KUP and HER were digested with papain (1/100 w/w) for 20 h in 5% formic acid at 37°C. The pFh was purified by gel filtration on Sephadex G-25 in 10% formic acid.

Isolation of Peptic Hinge Fragment (pFh)–IgG3 KUP and HER were digested with pepsin (1/100 w/w) for 20 h in 5% formic acid at 37°C. The pFh was purified by gel filtration on Sephadex G-25 in 10% formic acid.

Isolation of Pepsin Hinge Fragment (pFh)–IgG3 KUP and HER were digested with pepsin (1/100 w/w) for 20 h in 5% formic acid at 37°C. The pFh was purified by gel filtration on Sephadex G-25 in 10% formic acid.

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§ The abbreviations and fragment nomenclature used are: ABA, α-aminobutyric acid; PTH, phenylthiohydantoin; Δanapyl, 5-dimethylaminonaphthalene-1-sulfonyl; Me, allylamine, N,N-dimethyl-N-allylamine. ctFh, a 55-residue hinge fragment obtained by a-chymotrypsin digestion; pa ctFh, a 56-residue hinge fragment obtained by papain digestion followed by α-chymotrypsin digestion; pFh, a 64-residue hinge fragment obtained by pepsin digestion; tF(ab), a divalent F(ab)-type fragment obtained by trypsin digestion; ctFab, a monovalent Fab-type fragment obtained by a-chymotrypsin digestion; Cy1, a fragment containing the Cy1 domain and the hinge region of IgG3; Feh, a papain fragment which includes the Fc fragment and part of the hinge.
formic acid and was further purified by ion exchange chromatography on Whatman DE52 and eluted with 0.01 M phosphate buffer, pH 7.6.

Isolation of Fd' Fragments from IgG3 – Fd' fragments from IgG3 were isolated as previously described (6, 7). Briefly, the F(ab')2 peptic fragment was partially reduced and alkylated with [14C]iodoacetic acid before separating the Fd' fragment from light chains by gel filtration on Sephadex G-100 in 5 M guanidine HCl, 1 M acetic acid (6, 7).

Isolation of Fc fragments from IgG3 – Fc fragments were prepared as described previously by digesting IgG3 (20 mg/ml) in 0.1 M phosphate, pH 7.0) with papain (crystallized three times, Sigma) (1/100, w/w) for 45 min without reducing agents (15). Fc which contained the Fc region and part of the hinge was isolated by gel filtration on Sephadex G-15, followed by ion exchange chromatography on CM-Sephadex (15). The final preparations were often slightly contaminated with Fc fragments which are the end product of Fc if the papain digestion is continued (15).

Citraconylation – To prevent trypsin digestion at lysine residues, the lysine residues were modified by introducing citraconylic groups (15). Citraconylation was performed with the protein dissolved (5 to 20 mg/ml) in 6 M guanidine HCl, 0.006 M borate, pH 8.5, by adding citraconylic anhydride (Pierce) in 30 molar excess over the lysine content. The total volume of citraconylic anhydride was added over a period of about 15 min under constant stirring. The pH was kept at 8.0 to 8.5 by adding 2 M NaOH. After standing at 4°C for another hour, the samples were extensively dialyzed against 0.001 M NH4HCO3, pH 5.5, before lyophilization and storing at -30°C until use.

Reduction and Alkylation – Partial and total reduction and alkylation was performed as previously described (6).

Amino Acid Analysis – Amino acid analyses of peptides and proteins were performed after hydrolysis in 6 M HCl for 16 to 78 h (6, 17) either with a Beckman 120 or a Durrum D-500 automatic amino acid analyzer.

NH2-terminal Amino Acid and Amino Acid Sequence Analysis – NH2-terminal amino acid analysis was performed by the dansyl chloride method and identification was done on polyamide thin layer plates (15 × 15 cm) with standards plus samples on one side and samples on the other (15, 19). The plates were run in two dimensions in three different solvent mixtures (19). Automated amino acid sequence analysis was done on a Beckman 690C Sequencer using the Beckman Me, allylamine peptide program (10/29/74). Identification of amino acids was generally done by three and a minimum of two methods. Gas-liquid chromatography was performed with a 7620A Hewlett-Packard gas chromatograph on columns packed with Beckman-coated Superox 55786 (20/100, w/w) in layer chromatography or PTH-derivatives was done on polyamide plates (5 × 5 cm), in two dimensions (31). Standards were placed on one side and samples on the other. The amides of aspartic acid or glutamic acid were clearly discriminated by thin layer chromatography but for unknown reasons, PTH-Ser and PTH-Thr were difficult to detect. The remainder of the aliquot was hydrolyzed under vacuum with hydrochloric acid (HCl), and the products identified by amino acid analysis (22). All PTH derivatives were converted to the native amino acids except for PTH-Ser which was converted to alanine and PTH-Thr which was converted to a-aminoisobutyric acid (ABA) and PTH-Trp which was destroyed. Cysteine was detected by counting radioactivity of [14C]carboxymethylcysteine and thin layer chromatography. The runs were terminated when clear-cut identification of a residue was no longer possible.

High Voltage Electrophoresis – High voltage electrophoresis was done on Whatman No. 1MM or 3MM paper at pH 2.1, 3.5, or 6.5 by employing Savant (Hicksville, N.Y.) liquid-cooled electrophoresis tanks (23). Peptides were located by autoradiography if they contained 14C labeled cysteine or by staining a guide strip of the paper containing peptides were cut out for elution or further purification by electrophoresis at a different pH. Peptides were eluted from the paper with 6 M HCl if they were to be used only for amino acid analyses, or with 15% acetic acid if used for other purposes.

Diagonal Electrophoresis – Cysteine-containing peptides were specifically isolated by diagonal electrophoresis taking advantage of a change in their charge after performic acid oxidation (24).

Hinge Specific Antiserum – A Y3 hinge specific antiserum (anti-Fh) was raised in a rabbit by immunizing with Fab', fragments of IgG3 (HER) and absorbing with insolubilized Fab fragments of IgG3 (HER) (14).

RESULTS

Analysis of Chymotryptic Hinge Fragment, ctFh, from IgG3 Proteins of Different Allotypes – The ctFh fragment was isolated from the proteins HER, FRO, and JON as described under "Materials and Methods" by gel filtration in 5 M guanidine, 1 M acetic acid (Fig. 1a) followed by isoelectric focusing (Fig. 1b). The molecular weight of ctFh obtained by gel filtration (Fig. 1a) was 10,000 to 12,000. The amino acid composition was calculated (Table I) assuming 1 residue of leucine per chain (M, = 6,000). The amino acid compositions of ctFh fragments were identical for all IgG3 proteins regardless of allotype. The NH2-terminal amino acid sequence of completely reduced and 14C-alkylated ctFh was shown to be: Thr-Cys-Pro-Arg-Cys-Pro-Glu-Pro-Lys-Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys.

In addition, on the basis of radioactivity, [14C]carboxymethylcysteine was identified at position 20 and 26.

Isolation and Amino Acid Sequence of 15-Residue Peptide Which Is Repeated Three Times in ctFh Fragment – Since the complete sequence of ctFh was not obtained by automated sequencing analysis, the ctFh (from HER) was citraconylated and further digested with trypsin (1/50, w/w for 2 h at pH 8.5). The digest was then totally reduced and 14C-alkylated and gel-filtered on Sephadex G-15 in 5% formic acid (Fig. 2). The isolation of ctFh fragments from IgG3 - Fd' fragments from IgG3 were isolated as previously described (6, 7). Briefly, the F(ab')2 peptic fragment was partially reduced and alkylated with [14C]iodoacetic acid before separating the Fd' fragment from light chains by gel filtration on Sephadex G-100 in 5 M guanidine HCl, 1 M acetic acid (6, 7).
amino acid composition of material from each peak (I, II, and III) is shown in Table I. The amino acid sequence of material in Peak I was shown to be Cys-Pro-Glu-Pro-Lys-Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys-Pro-Arg. The repetitive yield for proline was 95% and that of Cys(Cm) based on counts per min was 82%. Thus, in order to account for the amino acid composition of ctFh (Table I), this 15-residue segment must be present three times in ctFh. Amino acid composition of material in Peaks II and III (Table I) indicated that they could be placed at the COOH-terminal and NH₂-terminal ends of ctFh, respectively (see below and Fig. 6), and that they contain 1 carboxymethylcysteine residue each. Consequently, based on the amino acid composition of ctFh (Table I) and the radioactivity (Fig. 2), ctFh fragment contains 11 S-S bonds and must contain a triplication of the peptide in Peak I.

Amino Acid Sequence of ctFh Fragment—In order to establish the complete primary structure of the ctFh fragment, intact IgG3 (HER) was digested with papain in the absence of cysteine. It has been previously shown (15) that papain under these conditions cleaves the hinge near the NH₂-terminal end as well as other places, and yields Fch fragments which contain the Fc region and part of the hinge (for purification, see “Materials and Methods”). The Fch preparation was further digested with α-chymotrypsin (1/50, w/w for 4 h at pH 7.0) and filtered on a Sephadex G-150 column under neutral conditions (Fig. 3a). Radial immunodiffusion analysis of the gel filtration fractions revealed a broad anti-Fh reactive peak which was divided into two fractions (1 and 2). After desalting on a Sephadex G-25 column in 10% formic acid, materials in the two pools were further separated by isoelectric focusing (pH 5 to 7) and both showed three peaks reacting with anti-Fh antiserum (Fig. 3, b and c). Based on their elution position on Sephadex G-150 and their amino acid analysis, it was shown that the material with pl 5.8 is identical with ctFh (Table I). The fragment with pl 4.7 has a lower molecular weight, which together with the amino acid composition (Table I, pa ctFh) indicate that this represents the COOH-terminal half of ctFh. This was proven by sequence analysis which yielded the sequence: Asp-Thr-Pro-Pro-Pro-Cys-Pro-Glu-Pro-Lys-Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys-Pro-Arg-Cys-Pro-Glu-Pro-Lys-Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys-Pro-Arg-

<table>
<thead>
<tr>
<th>Amino acid composition of different fragments and peptides of γ3 hinge</th>
<th>ctFh</th>
<th>pFh</th>
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<tr>
<td>Fig. 1</td>
<td>Fig. 2</td>
<td>Fig. 4</td>
</tr>
<tr>
<td>Peak I</td>
<td>Peak II</td>
<td>Peak III</td>
</tr>
<tr>
<td>Asp</td>
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<td>4.1</td>
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<tr>
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</tr>
<tr>
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<tr>
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<tr>
<td>Leu</td>
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</tr>
<tr>
<td>His</td>
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</tr>
<tr>
<td>Lys</td>
<td>3.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Arg</td>
<td>3.8</td>
<td>3.3</td>
</tr>
</tbody>
</table>

The numbers are given as moles of residues per mol of peptide.

Average of three different IgGs (HER, FRO, and JON).

Analysis after further purification by paper electrophoresis, pH 6.5.

Determined as cysteic acid after performic acid oxidation (26).

Determined as carboxymethylcysteine, thus giving too low values.

Fig. 2. Gel filtration on Sephadex G-15 in 5% formic acid of a trypsin digest of ctFh. The sample was totally reduced and alkylated with 11C]iodoacetic acid before being applied to the column. Column dimensions: 2.4 x 45 cm. Sample concentration: 3 mg/ml. Fraction volume: 1.8 ml. Thirty microliters from each fraction were taken for liquid scintillation counting.

Arrangement of S–S Bonds in ctFh Fragment—To study the arrangement of the S–S bonds in the hinge, diagonal electrophoresis was performed on a tryptic digest of ctFh (Fig. 4). All the cysteines appeared by this analysis to form interchain bonds based on the independent electrophoretic mobility and amino acid composition of the peptides (Table I, peptides 1, 2, 3, and 4). Since trypsin does not split between the 2 cysteines in peptide 2 which has the sequence: Ser-Cys-Asp-Thr-Pro-Pro-Pro-Cys-Pro-Arg-, these cysteines could form an intrachain S–S bond. To test this, material in Band 2 obtained after the first dimension (Fig. 4) was eluted and incubated with papain in order to split the Cys-Asp bond as noted when intact IgG3 molecules were digested to get pa ctFh. Then diagonal electrophoresis was run on the incubation mixture. Under a variety of incubation conditions (variations in incubation time, substrate enzyme ratio, and pH), no such cleavage was noted since either no digestion or random cleavage at many bonds occurred. Thus, while the first and third pair of cysteine residues of peptide 2 (see Fig. 6) could be involved in intrachain S–S bonds, this possibility is unlikely since the corresponding cysteines in the second replication are not, as shown by the appearance of pa ctFh fragments by treatment with papain without using reducing agents.

Characterization of COOH and NH₂ Termini of Hinge—In order to evaluate the possibility that there could be additional repeated sequences, COOH-terminal to the ctFh, two IgG3 myeloma proteins of different Gm types (HER and KUP) were digested with trypsin (1/100 for 2 h at pH 7.0) followed by gel filtration on Sephadex G-200 under neutral conditions. This yielded two components: one with a molecular weight of about 100,000 reacted with an antiserum to the Fab fragment and represented F(ab)₂; the other, reactive with anti-Fc had a molecular weight of about 50,000 and represented a disulfide-linked dimer of the COOH-terminal half of the heavy chain. After complete reduction and alkylation, the NH₂-terminal...
sequence of tFc was found to be: Cys-Pro-Ala-Pro-Glu-Leu-Leu-Gly-Gly-Pro-Ser-Val-Phe-Leu-Phe-Pro-Pro-Lys-Pro-Lys-Asp-Thr-Leu-Met-Ile-Ser. This indicates that trypsin cleaved the molecule immediately prior to the last cysteine of the hinge, and that following this the sequence became identical with the C2 of y3 (27). The finding rules out the possibility of further duplications COOH-terminal to the ctFh fragment. Since, based on the previous results from heavy chain disease proteins (10, 11), ctFh apparently does not include the whole hinge at the NH2-terminal side, it was necessary to obtain an overlap peptide stretching from the C-y1 to the hinge. To do so, Fd fragments of y3 (HER and KUP) were prepared (see "Materials and Methods") and treated with citraconic anhydride to mask lysine residues followed by trypsinization and gel filtration on Sephadex G-50 fine in 10% formic acid (Fig. 5). The main radioactive Peak IV contains the 15-residue hinge repetitive unit and also another cysteine-containing peptide which was further purified by paper electrophoresis at pH 6.5, followed by electrophoresis at pH 2.1. The amino acid composition of this peptide was: Asp, Thr, Glu, Pro, Gly, Cys, Val, Leu, His, Lys, Arg, and the amino acid sequence of the first residues was determined to be: Val-Glu-Leu-Lys-Thr-Pro-Leu-Glu-Asp-Thr. This peptide begins NH2-terminal to ctFh (see Fig. 6) since a 64-residue pH fragment obtained by pepsin digestion of intact IgG3 proteins HER and KUP (Table I, pHf) had an NH2-terminal sequence: Lys-Thr-Pro-Leu-Gly-Asp-Thr-Thr-His-10 Thr-Cys-Pro-Arg-Cys, thus placing its start at the 4th position of the above NH2-terminal hinge peptide. Furthermore, a tryptic peptide with the amino acid composition Val, Asp, Lys, Arg was recovered from Cy1-Fh (the purification of this fragment which contains the Cy1 domain and the hinge will described elsewhere). This peptide could correspond to the Val-Pro-Lys Arg sequence in y1 (30) which immediately precedes residue Val (215) and is probably located in the Cy1 domain NH2-terminal to the Val-Glu-Leu sequence (Fig. 6). These results taken together speak against the possibility of additional repetitions.

Determination of Number of Disulfide Bonds in Hinge—To confirm the results of the sequence studies, the number of S—S bridges in the hinge was estimated by subjecting the whole IgG3 molecule to partial reduction and 10% alkylation and separating the heavy and the light chain on Sephadex G-75 in 10% formic acid. Reduction was done at dithiothreitol concentrations ranging from 5 to 50 mM and alkylation was performed with a 20% molar excess of [14C]iodoacetic acid. The total content of radioactivity in the heavy chain peak was compared with that of the light chain peak. Assuming that 1 cysteine was alkylated in the light chain, the number of alkylated cysteines in the heavy chain was calculated as shown in Table II. At 10 mM dithiothreitol and higher, 12 cysteines in the heavy chain (11 from the hinge and 1 from H—L bond) were alkylated while at 5 mM dithiothreitol, one of the hinge S—S bonds was not reduced.

DISCUSSION

Previous studies have clearly demonstrated that some of the unique structural features of the y3 heavy chain are due to the presence of an extended hinge which is unusually rich in cysteine and proline residues as a result of a series of duplications (6–8, 10, 11). However, most of the work on the primary
The structure of the γ3 hinge has been done with defective heavy chain disease proteins and has not permitted a precise estimate of the number of cysteine residues, nor a clear-cut definition of the beginning and the end of the hinge in relation to the Cy1 and Cy2 domains (10, 11).

The present study elucidates the structure of the hinge region of intact γ3 heavy chains based on an analysis of four IgG3 myeloma proteins of varying Gm types, and resolves the discrepancies in earlier reports (6, 10, 17). The results provide clear-cut evidence that the hinge of intact γ3 chains contains 11 cysteine residues and that the unusual structure appears to be the consequence of quadruplication of a 15-residue segment as previously postulated (11). This proof of repetitive sequences was obtained from cRFh, a chymotryptic fragment which appears to include all the cysteine residues of the hinge, by digestion with trypsin at arginine residues. In addition, by using a variety of proteolytic enzymes, it was possible to show that there are no additional unexpected sequences at the COOH- and probably not at the NH2-terminal ends of the hinge and that in all probability the arrangement of the cysteine residues is in the form of a series of parallel interchain disulfide bonds.

Fig. 6 compares the linear sequence of the hinge of the γ3 chain to that of the γ1 chain of protein Eu (γ1) (29) and provides a possible interpretation of the findings. Starting after the valine which corresponds to residue 215 (γ1 numbering) there is an insertion of 47 residues, marked by the solid bars, which ends after the Cys-Pro at the end of Line 3. Following this, and starting with the glutamic acid corresponding to residue 216 (γ1 numbering), there is a striking homology between the remainder of the hinge and the hinge of γ1. Within this 62-residue region, one can identify a 15-residue repetitive sequence whose beginning can be placed at any point in the first Pro-Arg-Cys-Pro sequence.

Several hypotheses can be put forward to explain the structure of the hinge, all postulating tandem duplications of a basic DNA sequence or perhaps unequal crossing over between two hinge genes.

Based on the observed homologies, the hinge could have evolved by a tandem quadruplication of a 45-nucleotide region of DNA. The alternative which requires the smallest number of mutations, would code for the 15 residues corresponding to the region from Glu 216 to Pro 230 (γ1 numbering). As seen in Fig. 7, this would give identical sequences for the three COOH-terminal units and requires six substitutions and an insertion of 2 residues in the NH2-terminal segment. This interpretation is favored since it is consistent with, and perhaps explains, the recently described mutant IgG1 human myeloma proteins which have deletions of the region from residue 216 to 230 (30, 31), a mutant mouse myeloma protein MOPC 21, IF2 (32) which has a deletion of the Cy1 domain and begins normal sequence at a valine corresponding to Glu 216, and the finding in the majority of the γ and α heavy chain disease proteins of deletions of most of the variable regions and the whole Cy1 domain with resumption of normal sequence commonly at Glu 216 (33). Interestingly, one γ3 heavy chain disease protein (ZUC) has only retained the last 3 cysteines of the hinge beginning with the Glu-Pro-Lys sequence corresponding to residue 216, the rest of the hinge region being deleted together with the Cy1 domain (23). Several other points could also serve as the beginning of this basic subunit of DNA although there is no compelling evidence in favor of any of them.
These structural features of the hinge of \( \gamma_3 \), together with the observed homology of 60 to 70% between the hinge regions of other subclasses of IgG and an almost equal degree of homology with the hinge of the \( \gamma_1 \) chains might indicate that the 45-nucleotide DNA segment coding for Glu 216 to Pro 230 represents an ancient hinge "gene" which has mutated during the evolution of classes and subclasses. In the case of IgG3, as shown in this report, this region has quadruplicated, and in the human IgA1 subclass, a duplication has occurred (34). The explanation of the ZUC protein might then be a deletion of three of the DNA segments.

By several criteria, including diagonal electrophoresis and selective proteolytic digestion, no evidence for any intrachain disulfide bridges was obtained and therefore, it seems likely that all cysteine residues are involved in symmetrical inter-heavy chain bonds. Nevertheless, as previously reported, low concentrations of dithiothreitol (5 mM) apparently left one S–S bond unreduced (17). Due to the repetition in the hinge, it will be difficult to clarify which S–S bond is more resistant than the rest.

The previously observed homology of greater than 90% in the corresponding domains as compared to 60 to 70% homology in the hinge regions of the IgG subclasses suggests that forces leading to the evolution of human IgG subclasses may have affected the hinge preferentially. This relatively high mutation rate in the hinge could either indicate that the hinge can tolerate mutations without affecting its function, or might indicate the existence of forces favoring the structural and perhaps also the functional diversification of the hinge. A specific functional requirement might explain the conservation of the quadruplicated hinge unit of the \( \gamma_3 \) chain which otherwise would be expected to have been deleted during evolution.

Although the biological function of the hinge remains to be elucidated, its unique structure, apparently independent evolution, and conservation in most of the classes and subclasses of immunoglobulin suggest that it may serve some important functions.

Among several different functions for the hinge, one can suggest a possible role in modulating and coordinating the action of the binding (Fab) and effector (Fc) parts of the molecule, perhaps by transmitting a signal from the binding part to the effector part of the molecule when the antibody binds to antigen (35) or in providing a flexible or specific angle between the two binding sites of the molecule (36). Lastly, in

**Table II**

Radioactivity in light and heavy chain peaks of partially reduced and \(^{14}C\)-alkylated IgG3 (HER)

<table>
<thead>
<tr>
<th>Concentration of Dithiothreitol</th>
<th>Heavy Chain Peak</th>
<th>Light Chain Peak</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Total No.</td>
<td>No. of S–S bonds</td>
</tr>
<tr>
<td>M</td>
<td>cpm</td>
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<tr>
<td>0.005</td>
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<td>321,000</td>
<td>12.0</td>
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the absence of evidence localizing any specific function to the hinge, it seems possible that it can modify and influence the effector functions of the adjacent domains (37).

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