Interchain Disulfide Bonds of Goat Immunoglobulin G*

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ABSTRACT: A fragment of 82 residues which is a dimer of three peptides, cross-linked by five disulfide bonds, was isolated from peptic digest of goat immunoglobulin G. This fragment contained the interchain disulfide bonds, since one of these peptides was derived from the light chain and the other two from different sections of the heavy chain. The two peptides derived from the heavy chain were linked by an intrachain disulfide bond. The sequence of the peptide derived from light chain was Lys-Thr-Val-Lys-Pro-Ser-Glu-CMCys-Ser and the sequences of the peptides derived from heavy chain were Lys-CMCys-Pro-Lys-Gln-Pro-CMCys-Val-Arg-Gly-Pro-Ser-Val, and Thr-Ser-Thr-Pro-Pro-Lys-Val-Tyr-Pro-Leu-Thr-Ser-CMCys-CMCys-Gly-Asx-Thr-Ser-Ser. There is one disulfide bond between the two heavy chains and one disulfide bond between the heavy and light chains. The arrangement of the disulfide bonds between these peptide chains, as determined by the cystine diagonal method, is similar to that proposed for rabbit immunoglobulin G.

The direct isolation of a disulfide cross-linked fragment, containing sections of both heavy and light chains was previously reported for a human pathological IgG 1[1] (Steiner and Porter 1967) and rabbit IgG (O'Donnell *et al.*, 1970). We have used a similar approach for the isolation of such a fragment from goat IgG. A peptide from the light chain, which contained the half-cystine involved in a disulfide bond with the heavy chain, was isolated first. The presence of this peptide was then searched for in different fractions obtained from a peptic digest of intact IgG. The fraction containing this peptide emerged almost pure off a Sephadex G-50 column. After reduction and ^{14}C carboxymethylation this fragment was found to contain two peptides derived from the heavy chain, in addition to the light-chain peptide. One contained the heavy–light disulfide bond and corresponded to the beginning of the constant part of the heavy chain, and the other contained the heavy–heavy disulfide bond and corresponded to the hinge region. The two peptides derived from the heavy chain were linked by an intrachain disulfide bond. The evolutionary implications of sequence homologies at these regions of the molecule are discussed.

Materials and Methods

Goat IgG. Goat IgG was prepared from serum obtained from a single animal as described by Givol and Hurwitz (1969). Upon chromatography on DEAE-cellulose equilibrated with 0.0175 M sodium phosphate (pH 6.3), two peaks containing IgG were eluted, the first of which was the material used in this study.

Reduction and Carboxymethylation of Disulfide Bonds. Goat IgG was mildly reduced with DTT[2] (0.013 M) and alkylated with [^{14}C]iodoacetate. The radioactively labeled light and heavy chains were separated on a Sephadex G-75 column equilibrated with 1 M propionic acid. The isolated immunoglobulin chains were fully reduced in 6 M urea 0.25 M β-mercaptoethanol and carboxymethylated with nonradioactive iodoacetate, followed by dialysis against 0.1 M acetic acid, and lyophilization.

Reduction and Carboxymethylation of Cystine-Containing Peptides. Peptides containing intact disulfide bonds were reduced in buffer containing 0.01 M DTT, 0.1 M Tris-HCl, and 0.002 M EDTA (pH 8.2) for 90 min at 37°. Sulfhydryl groups were then alkylated with [^{14}C]iodoacetate (0.03 M final concentration) for 30 min at room temperature.

Purification of Peptide Mixtures. Mixtures of peptides were separated by one or more of the following procedures. Peptides were fractionated according to size by gel permeation on columns of Sephadex. In those cases where peptides contained intact disulfide bonds, all manipulations were performed in acidic buffers to minimize disulfide interchange (Ryle and Sanger, 1955). Peptide containing fractions were detected by absorbance at 225 nm. Chromatography on Dowex 50-X2 was performed with a volatile pyridine–acetate buffer system according to Schroeder (1967). High-voltage electrophoresis on Whatman No. 3MM paper was run at pH values of 1.9, 3.5, and 6.5. Descending paper chromatography of peptide mixture was performed employing the organic phase of 1-butanol–acetic acid–water (4:1:5, v/v). The dye orange G (Gurr, London, England) was used as a reference marker in paper chromatography. Staining of papers was done by dipping in 0.25% solution of ninhydrin in acetone. Radioactive peptides were located on paper by autoradiography with Kodak BB-54 X-Ray film for 12–24 hr, each spot containing 5000–20,000 cpm.

Amino acid analyses were performed as described by Moore and Stein (1963). Values reported are uncorrected for losses during a 20-hr hydrolysis period and are expressed relative to a suitable residue chosen as 1.0.

Amino Acid Sequence of Purified Peptides. Sequence determinations were done by the Edman-dansyl procedure (Edman, 1956; Gray, 1967). Dansylamino acid derivatives were identified by thin-layer chromatography on polyacrylamide sheets as described by Woods and Wang (1967). In certain cases these results were confirmed by the subtractive Edman procedure of Hirs *et al.* (1960).

Measurement of Radioactivity. Radioactivity was measured

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[1] The nomenclature used is based on that recommended by the World Health Organization (1966).

[2] Abbreviations used are: DTT, dithiothreitol; dansyl, 1-dimethylaminonaphthalene-5-sulfonyl; CM, *S*-carboxymethyl; PTH, phenylthiodydantoin; T, trypsin; C, chymotrypsin.

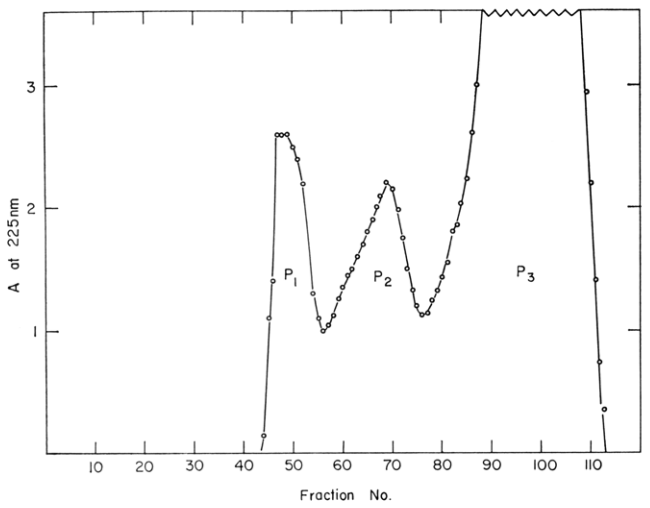


FIGURE 1: Fractionation of a peptic digest of goat IgG. A 300-mg portion of the digest was applied to a column (3 × 110 cm) of Sephadex G-75 equilibrated and eluted with 0.05 M acetic acid. The fraction volume was 7.5 ml. Peptide-containing fractions were detected by absorbance at 225 nm.

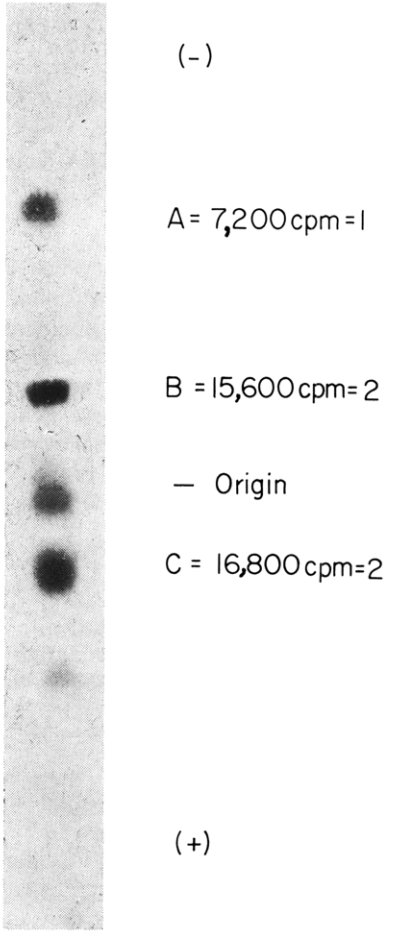


FIGURE 2: Autoradiography of the separation of [^{14}C]carboxymethylated peptides derived from fraction P_2 (see Figure 1). Reduced and alkylated P_2 was freed from reagents by passing through a Sephadex G-25 column equilibrated with 0.05 M NH_4HCO_3. The radioactive peptides, eluted in only one fraction, were electrophoresed at pH 6.5 for 30 min at 3 kV, and autoradiography was performed for 16 hr. Peptides A, B, and C contained 7200, 15,600, and 16,800 cpm, respectively.

in a Packard Tri-Carb liquid scintillator spectrometer, using dioxane containing scintillant (Bray, 1960) for liquid samples and toluene containing scintillant for paper samples.

Ultracentrifugation was performed in a Spinco Model E analytical ultracentrifuge. Measurement of sedimentation velocity was made at 59,780 rpm.

Enzymes and Reagents. Trypsin (L-(1-tosylamido-2-phenyl)-ethyl chloromethyl ketone treated), chymotrypsin, and pepsin were obtained from Worthington Biochemical Corp. Digestion of peptides with either trypsin or chymotrypsin was performed in 0.1 M NH_4HCO_3 for 3–4 hr at 37°. Digestion with pepsin was in 5% formic acid. DTT was purchased from Calbiochem and dansyl-Cl from Fluka. Polyacrylamide sheets were supplied by the Cheng Chin Trading Co. Ltd., Taiwan. Iodoacetic acid was recrystallized from ether–petroleum ether (bp 30–60°). ^{14}C-Labeled iodoacetate acid (21 mCi/mmole) was obtained from the Radiochemical Centre, Amersham.

Results

Carboxymethylcysteine-Containing Peptide from Light Chain. Goat IgG (400 mg in 15 ml of 0.2 M Tris-HCl, pH 8.2) was reduced with 30 mg of DTT for 2.5 hr at room temperature, followed by alkylation for 30 min with 135 mg of iodoacetate containing 20 μCi. The labeled protein (300,000 cpm) was dialyzed exhaustively against 1 M propionic acid and separated into heavy and light chains by gel permeation chromatography on a 3 × 90 cm column of Sephadex G-75 equilibrated with the same buffer. The distribution of radioactivity between heavy chain (215,000 cpm) and light chain (55,000 cpm) indicates one and four CM-cysteines on light and heavy chains, respectively. After lyophilization the CM-light-chain preparation was dissolved in 10 ml of 6 M urea–0.5 M $NaHCO_3$ and totally reduced by β-mercaptoethanol (0.25 M) for 3 hr at 37°, followed by alkylation with a neutralized solution of iodoacetic acid (final concentration of iodoacetate 0.35 M) for 30 min at room temperature. The reduced and alkylated light chain was dialyzed against 0.1 N acetic acid and lyophilized. The CM light chain (100 mg) was dissolved in 10 ml of NH_4HCO_3 (pH 8.7) and digested with 2 mg of trypsin (1:50 weight ratio) for 6 hr at 37°. The digestion mixture was acidified and applied to a 140 × 2 cm column of Sephadex G-25 fine, equilibrated with 1 N acetic acid. One major radioactive peptide was eluted in 80% yield, with only traces of other radioactive peaks. This peptide was further purified by Dowex 50-X2 chromatography (65% yield) and high-voltage electrophoresis at pH 6.5 (mobility *vs.* Asp = −0.19). In both procedures there was no evidence for more than one radioactively labeled peptide arising from the light chain.

The composition of this peptide was $CMCys_{1.1}$,$Thr_{0.9}$,-$Ser_{1.3}$,$Glu_{1.2}$,$Pro_{1.1}$,$Val_{0.8}$,$Lys_{1.0}$ and its sequence as determined by the dansyl-Edman technique was found to be: Thr-Val-Lys-Pro-Ser-Glu-CMCys-Ser. This sequence is identical with that recently described for the C-terminal peptide of light chain from bovine IgG (Beale and Squires, 1970) and it is homologous to the C terminus of the λ-type light chains of other species (Hood *et al.*, 1967). This peptide served in the following studies as a marker to detect the heavy-chain peptide linked to it by an interchain disulfide bond.

Cysteinyl-Containing Peptides from Heavy Chain Involved in Interchain Disulfide Bonds. Native IgG (300 mg in 15 ml

TABLE I: Amino Acid Composition of CM-cysteine-Containing Peptides Derived from Fragment P_2 by Reduction and Carboxymethylation.[a]

Residue	Peptide A	Peptide B_1	Peptide C
Lys	2.0	2.0	1.0
Arg		0.9	
CM-Cys	1.1	2.0	1.6
Asp			1.1
Thr	0.9		3.8
Ser	1.8	1.1	3.9
Glu	1.2	1.2	
Pro	1.1	2.8	2.4
Gly		1.0	1.0
Val	0.8	2.0	1.0
Leu			0.8
Tyr			0.7
Total	9	13	19
m =	0.37	0.0	−0.23

[a] Mobility (m) was calculated *vs.* the mobility at pH 6.5 of aspartic acid which was taken as −1 relative to the position of neutral amino acid.

of 5% formic acid) was digested with 25 mg of pepsin for 20 hr at 37°. The clear digest was fractionated on a 3 × 110 cm Sephadex G-75 column equilibrated with 0.05 N acetic acid (Figure 1). Samples from the various fractions were reduced and carboxymethylated with [^{14}C]iodoacetate followed by paper electrophoresis at pH 6.5. Only fraction P_2 yielded a peptide which after further tryptic digestion corresponded in its mobility and composition to the peptide containing CMCys derived from the light chain. Hence fraction P_2 was subjected to reduction and alkylation with [^{14}C]iodoacetate as described in Materials and Methods. The alkylated material was desalted on a Sephadex G-25 column and run on preparative scale at pH 6.5 electrophoresis followed by autoradiography (Figure 2). The autoradiography revealed a number of radioactive spots of which three designated A, B, and C were the most intense.

Comparison of the radioactivity of the different peptides showed that the ratio of radioactivity contained in peptide A:B:C was approximately 1:2:2. After elution from paper peptides, A and C were found to be pure. Peptide B was further fractionated by paper chromatography (butanol–acetic acid–water) to yield two components: peptide B_1 (migration *vs.* orange G = 0.35), which comprised 60% of the counts present in peptide B, and peptide B_2 which remained at the origin. Both have similar amino acid composition but only B_1 was sequenced. In several preparations the yield of fraction B relative to A varies somewhat from 2:1 and additional radioactive peptides were detected upon electrophoresis at pH 6.5. When eluted and analyzed the composition of these additional peptides was similar to that of peptide B_1, indicating either heterogeneous peptic splits or sequence heterogeneity of this area. The compositions of peptide A, B_1 and C are given in Table I. The overall yield of peptides A, B and C were determined from amino acid analysis of a sample from the paper eluate. This was found to be 20, 16, and 18% for A, B, and C, respectively. The yield of elution from paper was determined by comparison of the radioactivity present on the paper and in the eluate and was found to be around 50%. This implies that the yield of the peptides (corrected for losses during elution from paper) was around 0.8 mole/mole of IgG which is 40% of theoretical.

TABLE II: Amino Acid Composition of the Tryptic Peptides Obtained from Peptide B_1.[a]

	B_1T_1	B_1T_2	B_1T_3	B_1T_4
Lys				1.1
Arg		0.8	0.8	
CM-Cys		0.8	0.8	0.7
Ser	1.0			
Glu		1.0	1.0	
Pro	1.2	1.2	1.2	1.0
Gly	0.8			
Val	1.0	1.1	1.1	
m	1.3	1.6	1.8	2.1

[a] Mobility (m) is given as the ratio to the mobility of DNP-lysine at pH 1.9.

Peptide A. Its composition is similar to that of the peptide derived from CM light chain but it contains an additional lysyl residue. When peptide A (mobility *vs.* Asp at pH 6.5 = 0.37) was digested with trypsin it yielded one radioactive peptide (mobility *vs.* Asp = −0.17) which was identical in mobility and composition with the light-chain peptide described above, and a free lysine residue. Three steps of Edman-dansyl degradations showed the N-terminal sequence Thr-Val-Lys-Pro for this peptide. This and the presence of N-terminal lysine (by dansyl method) in peptide A indicated that peptide A was derived from the light chain. Its sequence must be identical with that of the peptide described in the previous section with an additional lysyl residue at its amino-terminal end.

Peptide B_1. Fraction B, as detected by paper electrophoresis at pH 6.5, contained several components. The large content of proline found by amino acid analysis (Table I) suggested that this peptide might have originated in the area of the heavy–heavy-chain disulfide bonds (the hinge region). This region is known to be very variable among immunoglobulin of different subclasses (Frangione *et al.*, 1969) which may also be the reason for the heterogeneity of this fraction here; two subclasses have been reported for goat IgG (Gray *et al.*, 1969) and they may differ in the sequence of this region.

Only the sequence of peptide B_1, isolated as a major component by paper chromatography, was determined and it is probably similar to other peptides of fraction B. The N-terminal sequence of peptide B_1 was found to be Lys-CMCys, both by dansyl-Edman and by subtractive Edman procedures. After tryptic digestion and electrophoresis at pH 1.9, four peptides were isolated, three of them radioactive. The composition and mobility of these peptides are given in Table II.

Peptide B_1T_1 has the sequence Gly-Pro-Ser-Val and it must be the C-terminal peptide since it contains no Arg or Lys. Peptide B_1T_4 has the sequence CMCys-Pro-Lys and it is the N-terminal peptide of B_1 from which the N-terminal lysine was removed by trypsin. Peptide B_1T_2 has the sequence Gln-Pro-CMCys-Val-Arg. The fourth peptide B_1T_3 has the

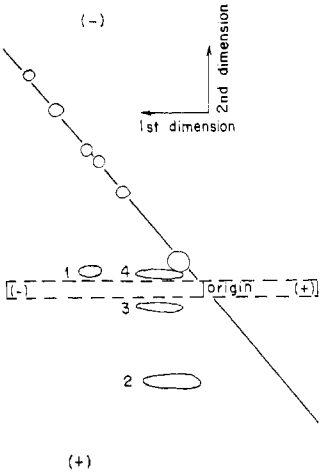
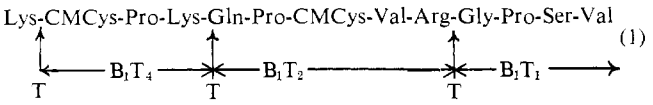


Figure 3: Cystine diagonal map of a tryptic digest of fragment P_2. The diagonal map was prepared by electrophoresis of the digest at pH 6.5 at 3 kV for 30 min. The paper was then oxidized with performic acid vapour and run in a second dimension at pH 6.5 The map was stained with ninhydrin followed by location of the arginyl peptides (Irreverre, 1965). Only one arginyl peptide (no. 1) defected the diagonal and it was unpaired with any other peptide. The composition of the off-diagonal peptides were (1) $Arg_{1.0}$,$Glu_{1.1}$,$Pro_{1.5}$,-$Val_{1.0}$,$CyA_{0.8}$ which is peptide B_1T_2, (2) $Asp_{1.0}$,$Thr_{2.4}$,$Ser_{3.4}$,$Pro_{1.0}$,-$Gly_{1.0}$,$Val_{0.9}$,$Leu_{1.2}$,$Tyr_{0.6}$,$CyA_{1.8}$ which is peptide CT_1, (3) $Lys_{1.1}$,-$Thr_{1.1}$,$Ser_{2.0}$,$Glu_{0.9}$,$Pro_{1.0}$,$Val_{1.0}$,$CyA_{1.0}$ which is the light chain peptide A, and (4) this peptide was not obtained in pure form and was probably contaminated by neutral peptides. However, the major residues were Lys, Pro, and CyA in a ratio of 1.0:0.9:1.0 indicating the presence of peptide B_1T_4.

same composition as B_1T_2 and probably the same sequence, but its mobility could have changed due to cyclization of glutamine. The identification of the N-terminal residue as Gln is based on the mobility of the peptide (neutral) at pH 6.5. Hence the sequence of peptide B_1 is as shown in eq 1.



(1)

This sequence is homologous to the sequence of the region involved in the heavy-chain disulfide bond in rabbit and human IgG but shows a gap between the Arg and Gly residues (Steiner and Porter, 1967; O'Donnell *et al.*, 1970; Givol and DeLorenzo, 1968).

Peptide C. Seven steps of dansyl-Edman degradations yielded the following N-terminal sequence: Thr-Ser-Thr-Pro-Pro-Lys-Val. Tryptic digestion yielded two peptides which were separated by electrophoresis at pH 6.5. Peptide CT_1 which contained all the radioactivity of peptide C and peptide CT_2 (Table III). Peptide CT_2 has the sequence Thr-Ser-Thr-Pro-Pro-Lys which is the N-terminal sequence of peptide C. The sequence of CT_1 was determined as follows.

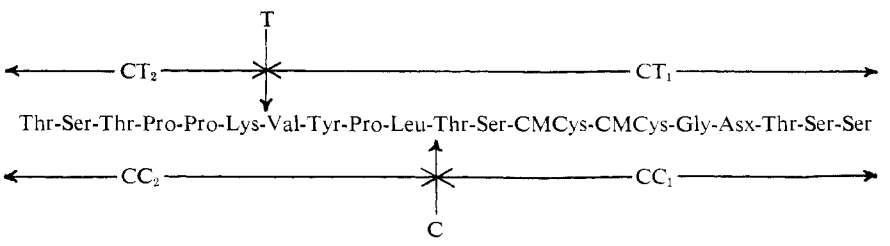
TABLE III: Amino Acid Composition of Tryptic and Chymotryptic Peptides Derived from Peptide C.[a]

	CT_1	CT_2	CC_1	CC_2
Lys		1.0		1.2
CM-Cys	1.4		1.6	
Asp	1.1		1.0	
Thr	2.0	0.9	2.0	1.0
Ser	3.1	1.9	3.0	2.1
Pro	0.9	1.6		3.0
Gly	1.2		1.0	
Val	0.8			1.1
Leu	1.0			1.0
Tyr	0.7			0.7
m (at pH 6.5)	−0.5	0.5		
m (at pH 3.5)			−0.8	2.1

[a] Mobility (*m*) was calculated relative to aspartic acid which was taken as −1, relative to the position of neutral amino acids.

Ten cycles of Edman degradations yielded the following sequence: Val-Tyr-Pro-Leu-Thr-Ser-CMCys-CMCys-Gly-Asx. The position of the CMCys residues was established mainly by measuring the radioactivity in the butyl acetate extracts containing the cleaved PTH derivatives. However, in view of the losses during many Edman degradation cycles it was necessary to establish unequivocally this sequence. Hence peptide C was digested with chymotrypsin to yield two peptides that were separated by electrophoresis at pH 3.5, a radioactive one CC_1 and a nonradioactive one CC_2 (Table III). Peptide CC_2 which contained the only lysyl residue of peptide C was further split by trypsin to yield a lysyl peptide which was identical with CT_2 and the peptide Val-Tyr-Pro-Leu which is the N terminus of CT_1. Peptide CC_1 (9 residues) was found to have the sequence Thr-Ser-CMCys-CMCys-Gly-Asx-Thr-Ser-Ser as determined by eight Edman degradation cycles. Hence the sequence of peptide C is as shown in eq 2. This peptide shows homology to the rabbit IgG heavy-chain peptide, which is present at the beginning of the constant part and is linked by an interchain disulfide bond to the light chain and by an intrachain disulfide bond to the hinge region of the heavy chain (O'Donnell *et al.*, 1970).

Arrangement of Disulfide Bonds in Fragment P_2. Homology considerations suggest that the arrangement of disulfide bonds in P_2 is similar to that present in rabbit IgG (O'Donnell *et al.*, 1970), namely, peptide A (derived from light chain) is linked to one of the cysteines of peptide C, and the other cysteine of peptide C is linked by an intrachain disulfide bond to peptide B_1. The other cysteine of peptide B_1 is involved



(2)

TABLE IV: Comparison of the C-Terminal Amino Acid Sequence of Goat Light Chain with Known Sequences Derived from λ-Type Light Chains.[a]

Human	Lys-Thr-Val-Ala-Pro-Thr-Glu-Cys-Ser
Goat	————Lys——Ser————
Bovine	————Lys——Ser————
Horse	Leu-Ser——Ser————Pro
Dog	Ala——Ala————
Pig	————Thr——Ser————Ala
Guinea pig	Ser-Lys————Ser————
Rabbit	Gly-Asx————

[a] Taken in part from Hood *et al.* (1967) and Beale and Squires (1970). Solid line indicates identity to the sequence of human light chain.

in the heavy–heavy bond. It was possible to establish this experimentally by locating the cysteic acid peptides in a cystine diagonal "map" (Brown and Hartley, 1963) performed on a tryptic digest of P_2. P_2 contains only one arginyl residue, which is present in the tryptic peptide Gln-Pro-CMCys-Val-Arg of peptide B_1. If the cysteine in this pentapeptide is involved in the heavy–heavy disulfide bond, it should appear on the cystine diagonal map as a single, unpaired peptide, whereas the rest of the disulfide-linked peptides (A, B_1T_3, and CT_1) should, after oxidation, fall on the same line of the diagonal. Figure 3 shows that this is indeed the case. Thus it can be concluded that the only possible arrangement which would account for all the half-cystine residues is that shown in Figure 4.

This arrangement could also be tested by sedimentation velocity analysis of the tryptic digestion of goat IgG. Digestion of goat IgG with trypsin (1:50, w/w) for 4 hr at 37° resulted in fragmentation of the 6.5S molecule to 3.5S fragments. This is compatible with the suggested arrangement of disulfide bonds and splitting of the Lys–Gln bond in the hinge region by trypsin (Figure 4), resulting in production of Fab and Fc fragments of 3.5S.

Discussion

Peptic digestion of goat IgG yields a large fragment composed of three peptides linked by disulfide bonds. It is very likely that they were present as dimers held together by the inter-heavy chain disulfide bond. Peptide A was identified as derived from the C terminus of the light chain and peptide B and C were found to be homologous to known region in the heavy chain. It should be emphasized that the localization of peptides B and C in the heavy chain is based only on homology with other species, since no sequence data on goat heavy chain are available. However, the arrangement of disulfide bonds was unequivocally established experimentally. On the basis of the arrangement of the disulfide bonds in fragment P_2 (Figure 4) it follows that the scheme for the interchain disulfide bonds in goat IgG is as depicted in Figure 5. It is shown that there is only one interchain disulfide bond between the heavy chains and the arrangement is similar to that found in rabbit IgG (O'Donnell *et al.*, 1970). The light chain peptide was the only CMCys-containing peptide present in CM light chain and this confirms previous identification of goat IgG light chain as belonging to the λ type

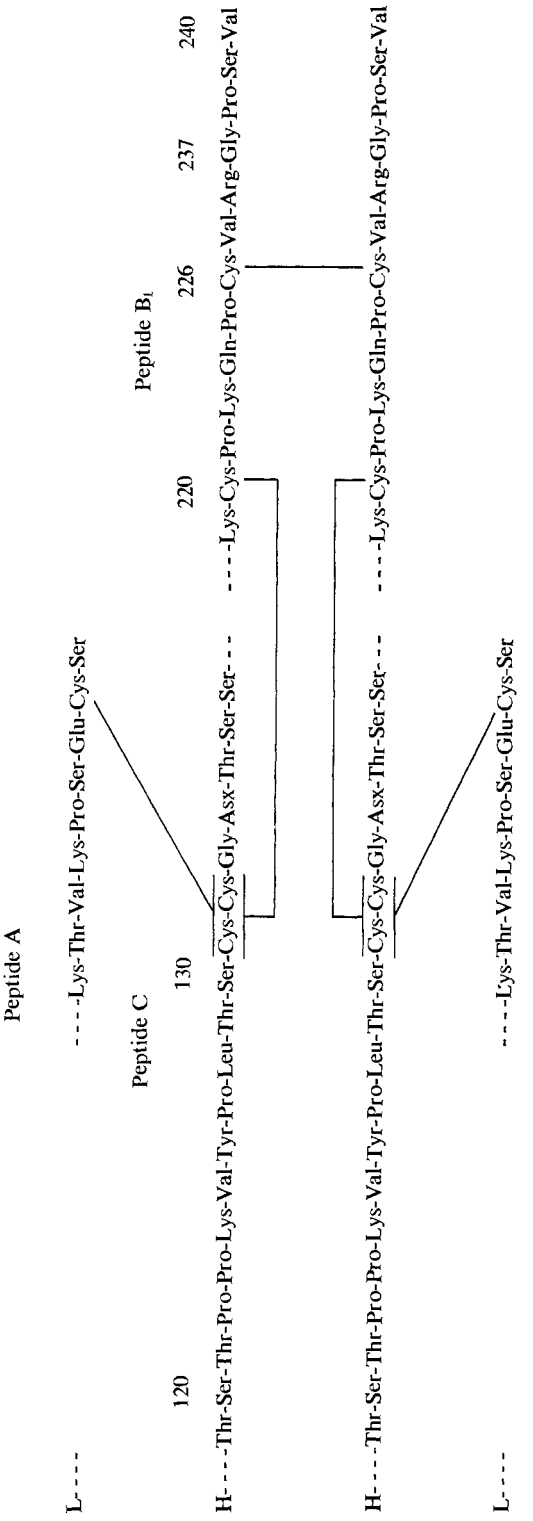


FIGURE 4: Summary of sequences of peptides derived from the peptic digest fraction P_2 of native goat IgG. Arrangement of disulfide bonds was determined by the cystine diagonal technique as described in text and in Figure 3. Residues are numbered from the N terminus based on the sequence of γ_1 chain Eu (Edelman *et al.*, 1969). L, light chain. H, heavy chain.

TABLE V: Comparison of the Sequences around the Position of Heavy–Light Disulfide Bond.[a]

	125	130	135
Goat	Pro——Lys——Tyr———	Thr-Ser-Cys-Cys-Gly———	Ser——
Rabbit	Ala-Pro-Ser-Val-Phe-Pro-Leu-Ala-Pro-	Cys-Cys-Gly-Asp-Thr-Pro-Ser	
Human γ_4	Gly———	Cys-Ser-Arg———	
Human γ_3	Gly———	Cys-Ser-Arg———	
Human γ_2	Gly———	Cys-Ser-Arg———	
Human γ_1	Gly———	Ser-Ser-Lys———	
Human μ		Leu-Val-Ser- Cys-Glx-Asx-Ser	

[a] Solid lines indicate identity to the sequence of rabbit IgG. Residues are numbered as in Figure 4. Data are from O'Donnell *et al.* (1970), Milstein and Pink (1970), and Kohler *et al.* (1970).

TABLE VI: Amino Acid Sequences of Peptides Derived from the Hinge Region of Immunoglobulin G Heavy Chains.[a]

	220	225	230	235	240
Human γ_1	Ser-Cys-Asp-Lys-Thr-His-Thr-Cys-Pro-Pro-Cys-Pro-Ala-Pro-Glu-Leu-Leu-Gly-Gly-Pro-Ser-Val				
Human γ_2	Cys-Cys-Val-Glu [■■■■] Cys———				
Human γ_3	——Cys——Thr-Pro-Pro-Pro-Cys——Arg———				
Human γ_3	Tyr-Gly-Pro-Pro-Cys———Ser——Phe———				
Rabbit	Thr-Cys-Ser——Pro [■■] ——Cys——— [■■■■]				
Goat	Lys-Cys-Pro—— [■■] Glu-Pro-Cys-Val-Arg [■■■■■■■■]				

[a] Comparison is made to the data tabulated by Milstein and Pink (1970). Residues are numbered as in Figure 4. Solid lines indicate identity with human γ_1 chain. Shaded blocks indicate gaps postulated to maximize homology.

(Givol and Hurwitz, 1969). Table IV compares some of the known sequences of λ-type light chain from different species.

The comparison of sequences of the heavy-chain peptides involved in interchain disulfide bonds shows some interesting phenomena. Table V compares the sequence of the peptides involved in the heavy–light bond. The sequence Cys-Cys in this peptide is of particular interest. It seems that there was a stepwise replacement of the two half-cystine residues during the evolution from rabbit to man. Three of the human IgG subclasses (γ_2, γ_3, and γ_4) still have half-cystine at that position which is linked to the light chain (Frangione *et al.*, 1969). However, γ_1 chain is devoid of both half-cystines and the heavy–light disulfide bond has migrated about 100 residues toward the hinge region. This might indicate that the gene for human γ_1 chain has evolved later than at least one of the genes of the other subclasses.

Table VI compares the sequence of the region involved in the heavy–heavy disulfide bond. This area (the hinge region) shows no homology to any of the main homologous domains of the heavy chain (Edelman *et al.*, 1969). This region also shows high variability in different subclasses, especially with respect to the number of half-cystines and to the presence of gaps (Frangione *et al.*, 1969). The large gap found in this area in goat heavy chain (Table VI) is particularly striking. It is suggested, therefore, that through mammalian evolution a process of addition of residues took place at that region in order to construct the link between the Fab and Fc fragments. The nature of the added residues in this region (preponderance of proline residues) is compatible with its function as a hinge for the mobility of the antibody fragments. The flexibility of the antibody fragments as well as some of its biological activity might very well be influenced by the structure of this area.

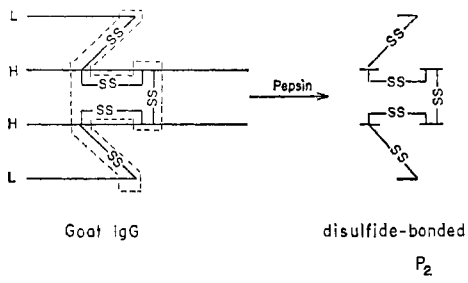


FIGURE 5: Arrangement of the interchain disulfide bonds in goat IgG and the schematic structure of P_2 derived from it by peptic digestion, according to Figure 4.

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Proton Magnetic Resonance Study of High- and Low-Spin Hemin Derivatives*

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ABSTRACT: Proton magnetic resonance spectra of several high-spin and low-spin hemin derivatives in deuterated dimethyl sulfoxide (Me_2SO-d_6) solution have been measured: (chloroprotoporphyrin IX)iron(III), (hemin); (chloro-2,4-diethyldeuteroporphyrin IX)iron(III), (mesohemin); (chlorodeuteroporphyrin IX)iron(III), (deuterohemin) and corresponding complexes with bromide, iodide, cyanide, and imidazole as axial ligands. Both the proton magnetic resonance and optical spectra show that a mixture of the chloride and Me_2SO complexes exist in hemin–Me_2SO solution at hemin concentrations of 0.01 M or greater, whereas at concentrations of 0.01 M or less in the bromo or iodo derivatives, only the Me_2SO complex is present. For the low-spin complexes (with cyanide and imidazole as axial ligands), the relative signs of the Fermi contact shifts are consistent with a π-electron mechanism for the contact interaction. However, the shifts for the 2,4 protons in the deuterohemin–Me_2SO complex are of the same sign and magnitude (approximately) as those for the α-methylene protons at the 2,4 positions in the mesohemin–Me_2SO complex. This last result suggests that other mechanisms (in addition to a π-electron one) may be involved in the contact interaction for the high-spin Me_2SO complexes. For the low-spin complexes, the proton magnetic resonance shifts yield an approximate estimate of 10^{-2} (positive) for the π-electron spin density at each peripheral carbon of the porphyrin ring.

The use of proton magnetic resonance to give information about metal–ligand bonding in paramagnetic transition metal complexes is well established (Eaton and Phillips, 1965). An analysis of the paramagnetic shifts can, in certain simple cases, provide a measure of the distribution of unpaired electrons in the ligands (McConnell and Chesnut, 1958; McConnell and Robertson, 1958; Kurland and McGarvey, 1970).

The work reported here, which deals with the proton magnetic resonance spectra of both high-spin ($S = 5/2$) and low-spin ($S = 1/2$) forms of hemin derivatives, was undertaken with several objectives: first, the proton magnetic resonance spectra of the hemin derivatives should be useful as models in understanding the proton magnetic resonance spectra of myoglobin, hemoglobin, and cytochromes; second, qualitative information about the relative stabilities of various hemin[1]–ligand complexes can be deduced from the proton magnetic resonance spectra; third, the paramagnetic proton magnetic resonance shifts can be interpreted (in principle) to give information about the electronic structure

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[1] Abbreviations used that are not listed in Biochemistry *5*, 1445 (1966), are: hemin, (chloroprotoporphyrin IX)iron(III); mesohemin, (chloro-2,4-diethyldeuteroporphyrin IX)iron(III); deuterohemin, (chlorodeuteroporphyrin IX)iron(III).