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Structure of Immunoglobulin A. Cysteine-Containing Peptides of the α Chain of an Immunoglobulin A1 Myeloma Protein†

Enrique Mendez,* Blas Frangione, and Edward C. Franklin

ABSTRACT: Studies of the cysteine-containing peptides of a completely reduced and alkylated heavy chain from a polymeric IgA1(κ) myeloma protein revealed an unusually high content of cysteine residues. At least 17 different radioactive cysteine residues were identified; since one additional cysteine-containing peptide may have been missed in this study, 18 is the minimum number of cysteine residues in the α chain. One of the cysteine residues formed the heavy–light disulfide bridge. There were at least two interchain heavy–heavy bridges, one involving a cysteine in the hinge and the other, a cysteine in a peptide disulfide bridged to the hinge. Two additional cysteine residues in the hinge form intrachain bridges with cysteine residues in the Fc and Fd fragments, respectively. While most of the remaining cysteine residues appear to be involved in intrachain bridges, it seems likely that some may be bridged to the secretory piece and J chain.

The IgA[1] fraction has several unusual characteristics: (1) unlike most other immunoglobulins, it frequently exists as a polymer linked to an unusual structural unit, the J chain by disulfide bridges (Morrison and Koshland, 1972); (2) in external secretions, IgA is bound to a unique subunit, the secretory piece. Here, too, in man, the bond is formed by disulfide bridges (Lamm and Greenberg, 1972); (3) the hinge region, while resembling that in most other classes of Ig in being rich in proline and cysteine residues, has several unusual properties which have been briefly described and will be elaborated in this and the accompanying report (Wolfenstein-Todel *et al.*, 1973). To try to understand some of these unusual characteristics of IgA, it seemed advisable to attempt to identify and localize all of the disulfide bridges of the molecule.

In recent years several studies have provided sequence data on a limited number of cysteine-containing peptides of the α chain. These include the carboxy-terminal peptide (Prahl *et al.*, 1971; Wolfenstein *et al.*, 1971; Kehoe *et al.*, 1973; Mendez *et al.*, 1973b), the hinge (Frangione and Wolfenstein-Todel, 1972), and a number of additional intra- and interchain cysteine-containing peptides (Wolfenstein *et al.*, 1971). More recently, Moore and Putnam (1973) have extended these studies and have described three additional cysteine-containing pep-

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[1] The nomenclature employed for the immunoglobulins follows that recommended by the World Health Organization, *Bull. W.H.O. 41*, 975 (1969).

tides from the α chain of a myeloma protein Ha.[2] The present study provides sequence data for virtually all of the cysteine-containing peptides of the α1 chain and together with the accompanying report (Wolfenstein-Todel *et al.*, 1973) emphasizes some of the unusual features of the α1 chain.

Materials and Methods

A human IgA1(κ) myeloma protein (Oso) was isolated by starch block electrophoresis at pH 8.6 (Kunkel, 1954). Polymeric and monomeric forms of the protein were purified by filtration on Sephadex G-200 in 0.3 M NaCl. Purity of the isolated proteins was determined by immunoelectrophoretic analysis using rabbit antisera to whole human serum and to γ, α, κ, and λ chains.

Partial and Complete Reduction and Alkylation. The purified protein (20 mg/ml) in 0.27 M Tris-HCl buffer (pH 8.2) was partially reduced with 5 mM dithiothreitol for 1 hr at room temperature under N_2. Unlabeled iodoacetic acid was added (100% excess) and allowed to incubate for 1 hr in the absence of light. The solution was dialyzed against distilled water following which the samples were freeze-dried. Heavy, light, and J chains were then separated by chromatography on Sephadex G-100 in 1 M acetic acid (3 × 130 cm column). The column effluent was monitored by absorbance of the fractions at 280 nm. The fractions containing heavy chains were freeze-dried and tested for purity by immunoelectrophoretic analysis using rabbit antisera against α and κ chains. The cold heavy chain was then completely reduced with 5 mM dithiothreitol in the presence of 7 M guanidine hydrochloride and alkylated with a twofold excess of [^{14}C]iodoacetic acid.

Enzyme Digestion. Labeled heavy chains were digested with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone trypsin (Worthington), enzyme–substrate ratio 1:20 w/w in 0.2 M ammonium bicarbonate (pH 8.3) (20 mg/ml), for 15 hr at 37°, and freeze-dried. After digestion, insoluble material was separated from the supernatant by centrifugation. The supernatant peptides were fractionated on a Sephadex G-50 column in 1 M acetic acid. The residue was also fractionated on Sephadex G-50. Two peptides were obtained which were further purified by chromatography on a Dowex AG 1-X2 column equilibrated with 1% pyridine. The elution of the column was achieved by stepwise increase in the concentration of acetic acid from 0.1–15 M. The large tryptic peptides were subjected to pepsin digestion (enzyme–substrate ratio 1:50 w/w) in 5% formic acid at a concentration of 20 mg/ml for 15 hr at 37°.

Purification of Radioactive Peptides. This was carried out by electrophoresis on Whatman No. 3 MM paper at pH 6.5 and 3.5. Following this the papers containing the radioactive peptides were oxidized with performic acid vapor (50 ml of formic acid plus 2.5 ml of 30% H_2O_2) for 3 hr in a desiccator at room temperature, to convert the carboxymethylcysteines present to the more acidic sulfone derivatives. The oxidized peptides were run again at pH 3.5. After a second oxidation the radioactive peptides were purified by electrophoresis at pH 2.1. All mobilities are given relative to the distance between ε-N_2ph-lysine and aspartic acid at pH 6.5.

Autoradiography. The localization of all radioactive peptides was carried out by autoradiography for 20 hr using Kodak Royal Blue X-ray film.

Polyacrylamide Gel Electrophoresis. Urea–disc electrophoresis was performed in polyacrylamide gels as described by Reisfeld and Small (1966). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was done under the same conditions as described by Maizel (1969).

Amino Acid Analysis. The peptides were hydrolyzed at 110° for 20 hr in high vacuum sealed tubes with 6 M HCl containing 0.1% phenol and analyzed in an automatic Beckman Model 121 amino acid analyzer equipped with high-sensitivity cuvettes and recorder.

N-Terminal Analysis and Edman Degradation. The N-terminal residues were allowed to react with dansyl chloride (Grey, 1967). The dansyl derivatives were separated by thin-layer chromatography (polyamide layers) as described by Woods and Wang (1967). To distinguish glutamic from aspartic acid, and threonine from serine, the peptides were subjected to chromatography with the third solvent described by Crawshaw *et al.* (1967) (ethyl acetate–methanol–glacial acetic acid, 20:1:1).

Edman degradation was done as described previously (Frangione and Milstein, 1968) using dansylation to mark the new amino-terminal residues. The dansyl derivative of carboxymethylcysteine cannot be detected with the solvents used because it does not separate from the 1-dimethylaminonaphthalene-5-sulfonic acid spot. The presence of [^{14}C]-carboxymethylcysteine was confirmed by the decrease in radioactivity or by thin-layer chromatography of the phenylthiohydantoyl (PhNCS) derivate.

Thin-Layer Chromatography. In order to distinguish between Glu and Gln, Asp and Asn, and CMCys, thin-layer chromatography (tlc) was employed. The thiazolinone derivatives of the amino acid residues were converted to the corresponding PhNCS derivatives by treatment with 0.2 ml of 1 M HCl at 80° for 10 min under nitrogen. The amino acid PhNCS derivatives were extracted into ethyl acetate and then assayed by tlc on silica gel sheets (Eastman Chromatogram 6060 silica gel) in solvent E described by Edman and Sjoquist (1956). The spots were visualized by ultraviolet spectral studies and stained with cadmium–ninhydrin reagent (100 ml of 1% w/v), ninhydrin in acetone, and 15 ml of 5% w/v cadmium acetate for 2 min at 110°. The PhNCS derivatives were also identified as such by gas chromatography using a Hewlett-Packard gas chromatograph.

Sequencer Determination. Automatic amino acid sequence determination was carried out with a Beckman Model 890 sequencer by the method of Edman and Begg (1967) using the peptide program. The amino acid PhNCS derivatives and the amino acid $Me_3SiPhNCS$ derivatives obtained by addition of 5 μl of *N,O*-bis(trimethylsilyl)acetamide to the PhNCS for 3 min at 80° were identified by gas chromatography with a Hewlett-Packard gas chromatograph (Pisano and Bronzert, 1969) (Hewlett Packard Co., Palo Alto, Calif.). Amino acid analyses of the hydrolyzed derivatives were also carried out.

Results

Separation of the Polypeptide Chain Components. Following partial reduction with 5 mM dithiothreitol and alkylation with cold iodoacetic acid the chains were separated by chromatography on a Sephadex G-100 column in 1 M acetic acid. The elution pattern shows the presence of four peaks (Figure 1). Analysis by polyacrylamide gel electrophoresis in urea indicated that the first peak contained a mixture of unreduced protein and probably aggregated J chain or H chain joined to J chain. The second and third peaks contained dimers and

[2] We thank Dr. Putnam for making a preprint of his manuscript available to us during the final stages of preparing this manuscript.

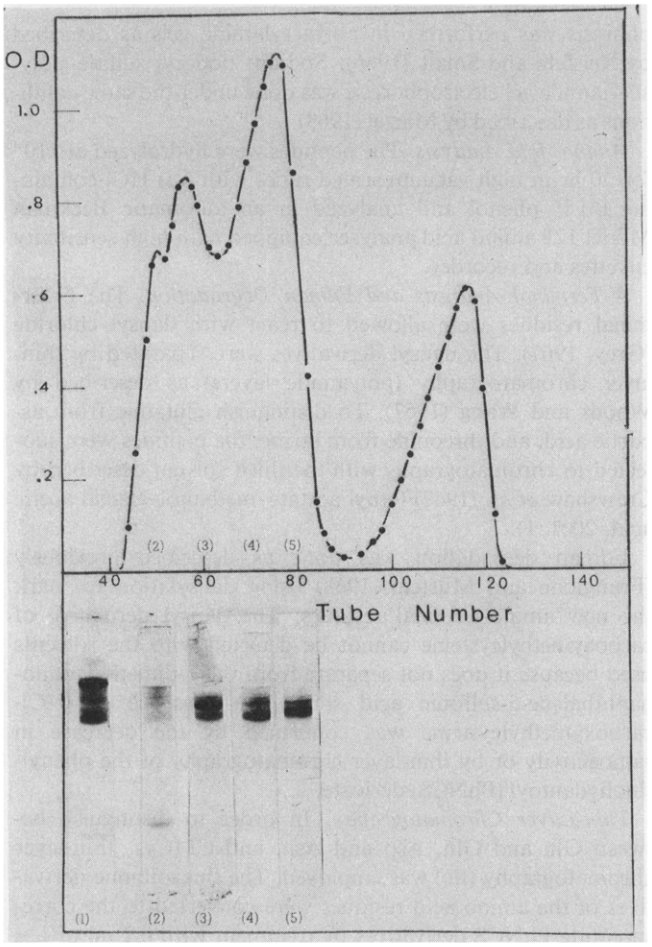

FIGURE 1: Fractionation of partially reduced and carboxymethylated myeloma protein Oso (IgA1 (κ)) on Sephadex G-100 in 1 M acetic acid (top), and polyacrylamide gels of material in each peak (bottom): (1) partially reduced and alkylated IgA1 (Oso); (2) polymers of heavy and J chains; (3) heavy-chain dimers; (4) and (5) heavy-chain monomers; (●) absorbance at 280 mn.

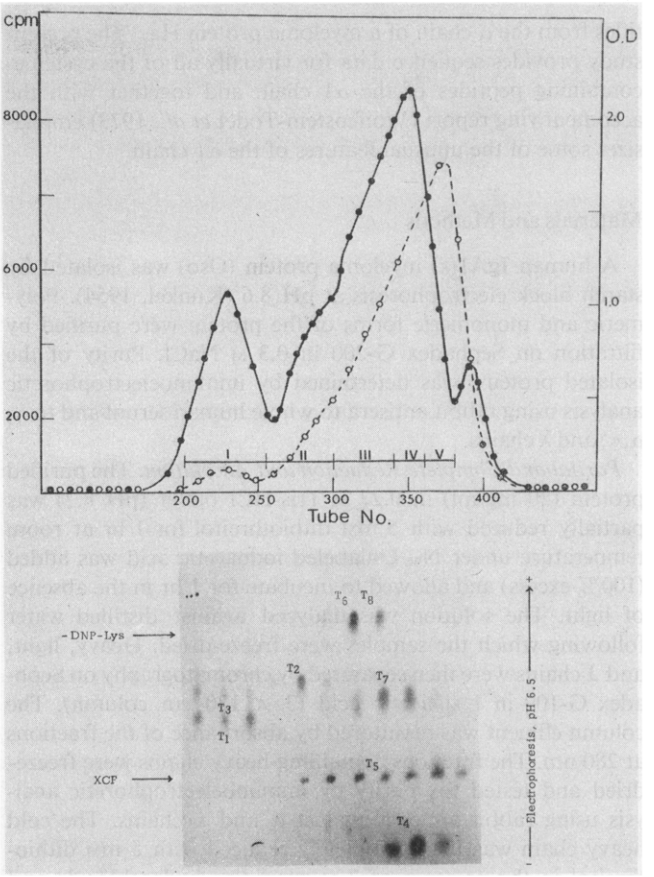

FIGURE 2: Fractionation of the soluble tryptic peptides of the α chain on Sephadex G-50 in 1 M acetic acid (top). Aliquots of fractions I–V were subjected to paper electrophoresis at pH 6.5 and the cysteine containing peptides were followed by autoradiography (bottom): (●) radioactivity; (○) absorbance at 280 nm; XCF Cyanol F F; -N_2ph-Lys indicates the position of neutral amino acid.

monomers of the H chain and the last peak of optical density contained the L chain. The J-chain region, which immediately precedes the L chain, can be detected only if reduction is performed with [^{14}C]iodoacetic acid since it can then be recognized as a separate peak because of its high cysteine content (Mendez *et al.*, 1972). The molecular weight of the α chain in polyacrylamide gel electrophoresis in sodium dodecyl sulfate was 61,000 and the N terminal was blocked. The amino acid analysis of the α chain is indicated in Table I.

TABLE I: Amino Acid Composition of the (Oso) α Chain.[a]

Lys	14.0	Gly	34.0
His	5.3	Ala	40.0
Arg	14.8	Val	35.5
CMCys	20.0	Met	4.1
Asp	40.0	Ile	5.2
Thr	66.0	Leu	60.0
Ser	53.8	Tyr	21.5
Glu	52.2	Phe	16.8
Pro	62.0		

[a] Compositions are reported as amino acid residues per molecule of peptide based on the molecular weight of 54,000 reported by O'Daly and Cebra (1971).

Fractions which were free of L and J chains and consisted of H-chain monomers and dimers were pooled and completely reduced with 5 mM dithiothreitol in the presence of 7 M guanidine hydrochloride and alkylated with [^{14}C]iodoacetic acid so as to open all the disulfide bridges.

Isolation of Tryptic Peptides from the α Chain. The labeled α chain was subjected to digestion with trypsin. After digestion, insoluble material was separated from the supernatant by centrifugation. The supernatant peptides were fractionated on a Sephadex G-50 column in 1 M acetic acid. Figure 2 shows the elution pattern of the soluble tryptic peptides. The eluted peptides were pooled into five fractions and an aliquot of each was subjected to paper electrophoresis at pH 6.5 and followed by autoradiography. The bottom of Figure 2 represents the distribution of the tryptic carboxymethylcysteine-containing peptides. At least eight main radioactive peptides were separated at this pH. In order to purify these peptides, each of the five pools was subjected to high-voltage electrophoresis on paper at pH 6.5, 3.5, and 2.1. The cysteine-containing peptides were identified by radioautography and eluted. The residue remaining after tryptic digestion was fractionated on a Sephadex G-50 column in 1 M acetic acid. The elution pattern is illustrated in Figure 3. The effluent fractions were divided into two main fractions. Aliquots of these fractions were subjected to high-voltage electrophoresis at different pH's and to paper chromatography. All of the labeled peptides remained at the origin, probably because of their large size. In order to purify these peptides, pools I and II were separately applied

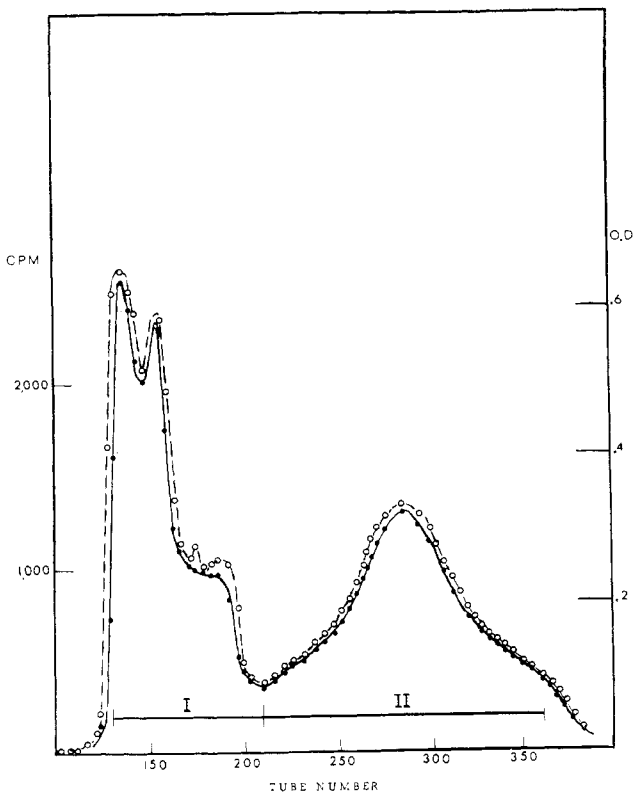

FIGURE 3: Separation on Sephadex G-50 in 1 M acetic acid of the precipitate remaining after tryptic digestion of a completely reduced and ^{14}C-carboxymethylated heavy-chain protein Oso. The pooled fractions are indicated on the figure: (○) absorbance at 280 nm; (●) radioactivity.

on a Dowex AG 1-X2 column equilibrated with 1% pyridine. Elution of the column was carried out in steps by increasing the concentration of acetic acid from 0.1 to 15 M. Figure 4 shows the elution pattern of this column. The top represents the elution pattern of the peptides coming from pool I and the bottom is the elution pattern of the peptides coming from pool II (Figure 3). Two main peaks, I-A and I-B, are obtained from the first peak and one main peak, II-A, is present in the second one.

Amino acid analysis and N-terminal residue determinations indicate that fraction I-A was completely pure and consisted of peptide T_9 (Table I), while the other remained contaminated. Because of their large size, the fractions from the precipitate were subjected to pepsin digestion following which the peptides were purified by paper electrophoresis at pH 6.5, 3.5, and 2.1.

Isolation of the Peptic–Tryptic Peptides of the κ Chain. The material in the light-chain region in Figure 1 had aspartic acid as N-terminal and an estimated molecular weight of 22,000 in sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The cold L chain in Figure 1 was completely reduced and alkylated with [^{14}C]iodoacetic acid followed by digestion with pepsin and trypsin. The carboxymethylcysteine-containing peptides were purified by paper electrophoresis at pH 6.5, 3.5, and 2.1. Four radioactive cysteine-containing peptides were found in addition to the cold carboxy-terminal cysteine peptide.

Characterization of the Cysteine-Containing Peptides from the α and κ Chains. The amino acid composition and mobilities of all carboxymethylcysteine-containing peptides from the α chains are shown in Table II and their sequences are indicated

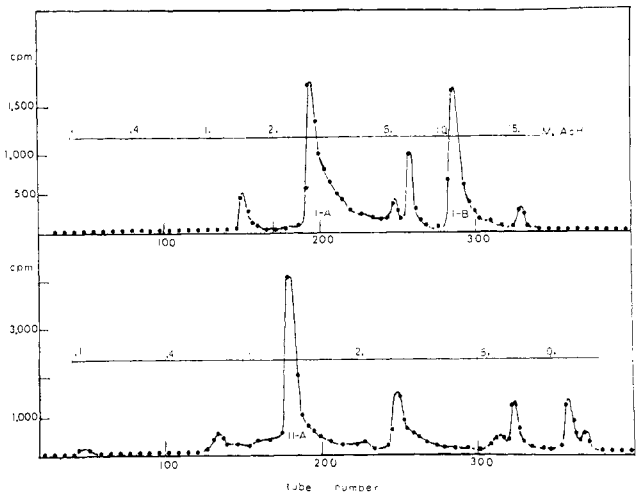

FIGURE 4: Chromatography of pools I and II (Figure 3). The peptides were fixed on a column (1.1 × 75 cm) of Dowex AG 1-X2 equilibrated with 1% pyridine. Elution was carried out in steps by increasing the concentration of acetic acid from 0.1 to 15 M in pool I (top) and from 0.1 to 10 M in pool II (bottom); (●) radioactivity.

in Tables III and IV. Their sequences are as follows.

PEPTIDE T_1 AND T_1a. Peptide T_1 (Table III) was found in very low yield and only the first eight residues were sequenced. Steps 4 and 5 were negative by the dansyl technique. Peptide T_1a (Table IV) is a soluble tryptic 22 residue peptide which was obtained in addition to T_1 and represents the C-terminal fragment of T_1 obtained as the result of splitting between Arg and Pro. The composition of this peptide based on the amino acid analysis is similar to peptide T_3 which was recently completely sequenced by Moore and Putnam (1973) and the sequence in the brackets is based on the sequence kindly provided by them (Table IV). Both peptides contained carbohydrate as identified by amino acid analysis.

PEPTIDE T_2. Since large amounts of this peptide were available, its sequence was established by manual and automatic Edman degradation. The results of the manual degradation are shown in Table IV. The N-terminal residue, Asn, was identified from the PhNCS derivative by gas chromatography, as well as by tlc. The amino acid analyses, N-terminal residues, and mobilities of the peptic peptides prepared from T_2 are given in Table V, and the amino acid sequences of these peptides are listed in Table IV. A closely related soluble peptide, T_2a, which was obtained in addition to T_2, added four residues to the sequence at the carboxy terminus. It overlapped peptides T_2Pe and T_2Pb. In the case of T_2Pb, the cysteine was identified by a decrease in radioactivity. The automatic Edman degradation of the peptide yielded unambiguous results for 16 steps. The assignment of Asp step 11 and Gln step 25 is based on the mobility of peptide T_2Pc and T_2Pb, respectively. The sequence of this peptide is identical with peptide T_2 of Moore and Putnam (1973) with the exception of our finding a serine residue in the penultimate position.

PEPTIDE T_3. This peptide contains the hinge region. It is the longest soluble CMCys tryptic peptide and contains an exceptional concentration of 14 proline residues and 3 Cys residues. The amino acid composition, mobility, and NH_2 residues of the peptic peptides are listed in Table VI. The amino acid sequence of T_3Pb which starts with residue 9 has been published previously (Wolfenstein *et al.*, 1971). The amino acid sequences of the extended peptide and its peptic derivatives are shown in Table IV. Carbohydrate was detected by amino acid analysis. The N-terminal residue in T_3 and T_3Pa

TABLE II: Amino Acid Composition of Carboxymethylcysteine Peptides Obtained from Completely Reduced and Alkylated IgA1-(κ) Myeloma Protein (Oso).[a]

	Supernatant												Precipitate				
Peptides[b]	T_1[c]	T_1a	T_2	T_2a	T_3	T_4	T_5	T_5a	T_5b	T_6	T_7	T_8	T_9	T_9-Pa	T_9-Pb	T-Pa[d]	T-Pb
Lys			1.0	1.1		0.9	1.9	1.0	1.0	0.8	1.0	0.7	1.0				
His	0.9				1.4	1.0	0.9	0.6	1.0	0.7		0.4					
Arg	1.6	0.8			0.8							0.8					
$CMCysSO_2$[e]	0.6	0.3	0.5	0.3	1.7	0.4	0.4	0.5	0.4	1.4	0.5	0.6	1.9	0.8	0.8	0.9	1.0
Asp	1.8	1.8	2.8		2.2		1.0	1.0	1.2	1.6		3.6	2.6	1.8			
Thr	2.8	2.7	3.7	1.8	5.9	1.2	1.9	0.9	1.0		2.7	2.4	1.6	0.9		2.0	1.0
Ser	2.0	1.0	4.4	0.7	5.8	1.1	1.0	1.0	1.0	2.7	1.2	1.1	2.1	2.3		1.0	
Glu	2.2	2.1	3.2	1.0	1.1		2.2	1.2		1.0	1.1	1.4	2.4	1.0			
Pro	1.3	0.9	3.4	1.4	14.1		0.9	0.9		2.0	1.1	1.4	3.8	1.1			
Gly	2.3	2.0	2.4	1.3			2.1	2.1	2.1	3.0		1.1	3.2			0.9	
Ala	2.0	2.0	3.2	1.9			2.1	1.1		1.0	2.3	2.9	1.5		0.9		
Val					3.2	2.3	0.9	1.0	1.1	2.0		2.6	2.4				
Met							0.8	0.7	0.7			1.4					
Ile													0.9				
Leu	9.0	6.8	3.8	1.9			1.9	1.9		1.7		1.4	3.6	1.3	1.3		0.8
Tyr			0.6							0.7	0.3		1.0				
Phe			1.0				1.8	0.5	1.4		1.1	1.7	2.0			1.0	
N-Terminal	Leu	Pro	Asn	Thr	His	Ser	Lys	Lys	Lys	Asp	Thr	Asp	Ser	Ser	Ala	Thr	Leu
Mobility pH 6.5[f]	0.20	0.4	0.32	N	0.3	−0.18	N	0.22	N	0.44	0.22	0.44	ND[h]	0.53	0.53	0.45	0.55
CHO[g]	+	+			+						+						

[a] Compositions are reported as moles of amino acid per mole of peptide. [b] Hydrolysis for 20 hr. [c] T = tryptic. [d] TP = tryptic-peptic. [e] Detected on amino acid analyzer. [f] Mobilities are given relative to the distance between ε-N_2ph-lysine and aspartic acid. [g] $CMCysSO_2$, carboxymethylcysteine sulfone. [h] ND = not done.

was difficult to identify precisely since it was negative by the dansyl technique. Although a decrease in radioactivity was noted after cleavage of the first residue from T_3, this did not happen in T_3Pa. Definitive identification of His at position one and Pro at position two was achieved by tlc of the PhNCS derivatives of peptide T_3 (Tables III and IV). The extra proline and cysteine in T_3Pa (Table IV) probably represent small amounts of contaminants.

PEPTIDE T_5. This peptide has 21 residues and Lys as N-terminal and C-terminal residues. Two other soluble tryptic peptides, T_5a and T_5b, with Leu and His as C-terminal residues were also found and probably represent the results of splitting of the T_5 peptide by a chymotrypsin contaminant between Leu and Ala and His and Glu, respectively. The sequence of the peptide is listed in Table IV and the amino acid composition mobility and NH_2-terminus of the related tryptic peptides and the peptic derivatives are shown in Table VII. This peptide contains the four residue T_7Th3 peptide of Moore and Putnam (1973).

PEPTIDE T_6. The sequence of this peptide was established by manual dansyl-Edman degradation. The NH_2-terminal residue Asp was determined by tlc and gas chromatography. The peptide contained a high content of cysteine residues and carbohydrate was detected by amino acid analysis. The complete sequence obtained by studying several peptic peptides is shown in Table IV and the mobilities, amino acid composition, and NH_2-terminals are shown in Table VIII.

PEPTIDE T_9. This peptide containing two cysteine residues was obtained in relatively low yield from the insoluble residue as peak IA (Figure 4) and contains the HL bridge which has been partially sequenced previously (Wolfenstein *et al.*, 1971). Its sequence is shown in Table IV. After pepsin digestion peptide T_9Pa and T_9Pb were isolated. T_9Pa contains the amino-terminal cysteine previously shown to be involved in the HL bridge while T_9Pb must be derived from the carboxy end of the peptide.

OTHER PEPTIDES. The amino acid sequence of peptides T_4, T_7, and T_8 was established by dansyl-Edman procedure and the partial sequences are indicated in Table III and IV. Only T_7 was shown to contain carbohydrate on amino acid analysis.

Two other CMCys peptides (TPa and TPb) were isolated from the residue by digestion of the impure peaks from the Dowex column (Figure 4) with pepsin.

Peptide TPa was recovered in low yield and could only be sequenced for two steps. TPb is probably derived from the region Leu-Thr-Cys-Thr-Leu of peptide T_1 or the subtilisin peptide S in Table III.

LIGHT-CHAIN PEPTIDES. Table IX lists the mobilities and amino acid sequence of the cysteine-containing light-chain peptides. P-T_1 and P-T_2 are derived from the variable and P-T_3 and P-T_4 from the constant region intrachain disulfide bridges, respectively.

Discussion

Studies of the intra- and interchain disulfide bridges have provided abundant information about the structure of all classes of immunoglobulins in many species. To date, however, little is known about the total number and location of the disulfide bridges of IgA (Wolfenstein *et al.*, 1971; Moore

and Putnam, 1973). Recently, eight cysteine-containing peptides were isolated from the J chain (Mendez *et al.*, 1973a) and, in the present study, four carboxymethylcysteine-containing peptides involved in intrachain bridges were isolated from the κ chain (Table IX). The present study suggests that the α chain appears to contain at least 18 cysteine residues, a number which are in excess of that noted for each of the 4 subclasses of IgG (Frangione *et al.*, 1969; Milstein and Frangione, 1971).

While the location and arrangement of some of these CM-Cys peptides have been defined, those of several others remain to be determined. Peptide T_3 has extended the sequence of the hinge by adding eight residues which were not found in the previously characterized peptic–tryptic peptide which had three cysteine residues and was rich in proline, serine, and threonine. Recently Frangione and Wolfenstein-Todel (1972) described that this peptide contained two identical sequences of seven residues repeated at least twice, Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser. It seems possible that there is another identical fragment with three substitutions in the sequence: Thr-*Val*-Pro-*Cys*-Pro-*Val*-Pro-Ser.

The hinge of IgA is unusual in apparently being bridged to two other peptides by disulfide bridges. Structural studies of the hinge peptide (Wolfenstein-Todel *et al.*, 1973) describe the location and structural relations of the three cysteine residues of the hinge. Diagonal maps of a subtilisin digest of the hinge and its disulfide-bridged peptides showed that the first cysteine of the hinge is bound to the cysteine contained in peptide T_2, while the last cysteine residue is bound to one of the cysteine residues of peptide T_6. Since it has previously been demonstrated that the C-terminal cysteine of the α chain is probably not involved in an interchain bridge (Prahl *et al.*, 1971), and that the hinge peptide can exist as a dimer (Abel and Grey, 1971), it seems certain that the third cysteine residue in the hinge is involved in interchain bonding while the other two cysteine residues appear to be involved in intrachain bridges. Thus, it would appear that only one of the three cysteine residues in the hinge is involved in interchain bridging, and that at least one additional inter-heavy–heavy bridge is located in peptide T_6 which is bound to the hinge by a disulfide bridge (Wolfenstein-Todel *et al.*, 1973).

Homology studies with other immunoglobulins indicated that one of the tryptic peptides T-Pa, Thr-Cys (Thr, Phe, Ser, Gly), is similar to the peptide involved in the first intrachain bridge of the Fd region in position 20 (Wikler *et al.*, 1969; Press and Piggot, 1967). Based on homology considerations, its mate has not been recovered so that it seems likely that at least one additional cysteine peptide remains to be identified.

Another tryptic peptide, T_9 with 33 residues and two cysteine residues, contains two peptic peptides. One is T_9Pa, Ser-Leu ($CMCys_{0.8}$,$Asp_{1.8}$,$Thr_{0.9}$,$Ser_{1.3}$,$Glu_{1.0}$,$Pro_{1.1}$,$Gly_{1.0}$), the peptide involved in the inter-heavy–light disulfide bridge (Wolfenstein *et al.*, 1971). The other, T_9Pb, Ala-Cys-Leu, probably contains the cysteine residue of the first intrachain bridge of the Fdc region based on homology. Unfortunately, it was not possible to sequence the region between the H–L and the next bridge. Since diagonal map studies indicated that T_9Pb, Ala-Cys-Leu, is bridged to peptide T_4, it seems likely that T_4 contains the other cysteine residue of the Fdc region (unpublished results).

The amino acid sequence around the cysteine residue of peptide T_5 has strong homology with the sequence around the cysteine of the last intrachain bridge of several immunoglobulin heavy chains (Edelman *et al.*, 1969). The first seven residues extend the sequence of the 40 residue peptide derived

TABLE III: Amino Acid Sequence of Carboxymethylcysteine Peptides Isolated from a Completely Reduced and Alkylated α Chain of an IgA1(κ) (OSO) Myeloma Protein.

Peptides	
T_1	Leu-Ser-Leu(His,Arg)Pro-Ala-Leu($CMCys_{0.6}$,$Asp_{1.8}$,$Ser_{1.0}$,$Glu_{2.2}$,$Gly_{2.3}$,$Ala_{1.0}$,Leu_6)Arg
T_2	Asn-Phe-Pro-Pro-Ser-Glu-Asp-Ala-Ser-Gly-Asp-Leu-Tyr-Thr-Thr-Ser-Ser-Glx-Leu-Thr-Leu-Pro-Ala-Thr-Gln-Cys-Leu-Ala-Gly-Ser-Lys
T_3	His-Pro-Thr-Asn-Pro-Ser-Glu-Asp-Val-Thr-Val-Pro-Cys-Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-Pro-Ser-Thr-Pro-Pro-Thr-Pro(Ser,Pro,Ser)Cys-Cys-His-Pro-Arg
T_4	Ser-Val-Thr-Cys-His-Val-Lys
T_5	Lys-Gly-Asn-Thr-Phe-Ser-Cys-Met-Val-Gly-His-Glu-Ala-Leu-Pro-Leu-Ala-Phe-Thr-Glu-Lys
T_6	Asp-Leu-Cys-Gly-Cys-Tyr-Ser-Val-Ser-Ser-Val-Leu-Pro-Gly-Cys-Ala-Glu-Pro-His-Gly-Asx-Lys
T_7	Thr-Phe-Thr-Cys-Thr-Ala-Ala-Tyr-Pro-Glu-Ser-Lys
T_8	Asx-Glx-Val-Phe-Leu-Ala-Cys-Val-Thr($Asp_{2.6}$,$Thr_{1.4}$,$Ser_{1.1}$,$Pro_{1.1}$,$Gly_{1.1}$,$Ala_{1.9}$,$Val_{0.6}$,$Met_{1.4}$,$Leu_{0.4}$,$Phe_{0.7}$,$Lys_{0.7}$,$His_{0.43}$,$Arg_{0.8}$)
T_9	Ser-Leu-Cys-Ser-Thr-Glx-Pro-Asx-Gly-Asx(CMCys$_{0.9}$,$Asp_{0.6}$,$Thr_{0.6}$,$Glu_{1.4}$,$Pro_{2.8}$,$Gly_{2.2}$,$Ala_{1.5}$,$Val_{2.4}$,$Leu_{2.6}$,Phe_2,$Ile_{0.91}$,$Tyr_{1.0}$)Lys
T_9Pb	Ala-Cys-Leu
TPa	Thr-Cys($Thr_{1.0}$,$Ser_{1.0}$,$Gly_{0.9}$,$Phe_{1.0}$)
TPb	Leu-Thr-Cys
COO^- [a]	Ala-Glu-Val-Asp-Glu-Thr-Cys-Tyr
S[b]	Thr-Cys-Leu-Ala-Arg

[a] Taken from Wolfenstein *et al.* (1971). Not isolated in the present study. [b] Subtilisin peptide. Not isolated in the present study (unpublished results).

TABLE IV: Amino Acid Sequence of Cysteine-Containing Peptides from α Chain of Protein Oso.

[a] Residues 4 and 5 not identified. Reported as His-Arg in 1 peptide by Moore and Putnam (1973). Sequence in brackets arranged to correspond to T1 peptide of Moore and Putnam.
[b] Wolfenstein *et al.* (1971).

from the carboxy-terminal cyanogen bromide fragment of an α chain (Kehoe *et al.*, 1973). Except for the C-terminal lysine, which was reported by them to be arginine, there was complete identity. If this difference can be demonstrated in other α chains, it may reflect the existence of a genetic polymorphism. Diagonal map studies (unpublished results) indicated that T_5 is bound to a subtilisin peptide, Thr-Cys-Leu-Ala-Arg, which must therefore represent the amino-terminal cysteine

TABLE V: Peptides Isolated from Peptide T_2 after Digestion with Pepsin.

Peptide	Mobility[a]	Composition	NH_2-Terminal
T_2Pa	0.4	$Asp_{2.2}$,$Ser_{0.9}$,$Glu_{1.0}$,$Pro_{2.3}$,$Phe_{1.0}$	Asp
T_2Pb	0.4	$CMCys_{0.4}$,$Thr_{0.8}$,$Glu_{1.0}$,$Pro_{1.5}$,$Ala_{0.7}$,$Leu_{1.0}$	Pro
T_2Pc	0.46	$Asp_{1.3}$,$Ser_{1.0}$,$Gly_{0.9}$,$Ala_{1.0}$,$Leu_{0.9}$	Ala
T_2Pd	0.52	$Asp_{2.7}$,$Ser_{2.1}$,$Glu_{1.1}$,$Pro_{2.0}$,$Gly_{1.3}$,$Ala_{0.8}$,$Leu_{1.1}$,$Phe_{0.7}$	Asp
T_2Pe		$Thr_{1.0}$,$Leu_{2.3}$	Leu
T_2Pf	0.39	$Asp_{1.0}$,$Ser_{1.1}$,$Glu_{1.1}$,$Pro_{1.8}$,$Phe_{0.5}$	Asp

[a] Mobilities are given relative to the distance between ε-N_2ph-Lys and aspartic acid.

TABLE VI: Peptides Isolated from Peptide T_3 after Digestion with Pepsin.

Peptide	Mobility[a]	Composition	NH_2-Terminal
T_3Pa	0.2	$CMCys_{0.2}$,$Asp_{1.8}$,$Thr_{1.3}$,$Ser_{1.3}$,$Glu_{0.8}$,$Pro_{2.7}$,$His_{0.6}$	His
T_3Pb	0.14	$CMCys_{1.5}$,Thr_5,Ser_5,Pro_{12},$Val_{3.2}$,$His_{1.5}$,$Arg_{1.0}$	Val

[a] Mobilities are given relative to the distance between ε-N_2ph-Lys and aspartic acid.

TABLE VII: Peptides Isolated from Peptide T_5 after Digestion with Pepsin.

Peptide	Mobility[a]	Composition	NH_2-Terminal
T_5Pa	N	$Lys_{1.0}$,$Asp_{1.1}$,$Thr_{1.0}$,$Gly_{1.1}$,$Phe_{0.8}$	Lys
T_5Pb	0.38	$His_{0.6}$,$Cys_{0.4}$,$Ser_{0.9}$,$Glu_{1.1}$,$Gly_{1.3}$,$Ala_{0.9}$,$Val_{0.9}$,$Met_{0.9}$,$Leu_{0.9}$	Ser
T_5Pc	0.24	$Lys_{0.9}$,$His_{0.8}$,$Cys_{0.5}$,$Asp_{0.9}$,$Thr_{0.9}$,$Ser_{0.9}$,$Glu_{1.0}$,$Gly_{2.0}$,$Ala_{1.0}$,$Val_{1.0}$,$Met_{0.5}$,$Leu_{1.2}$,$Phe_{1.0}$	Lys
T_5Pd	N	$Pro_{1.3}$,$Ala_{0.7}$,$Leu_{0.9}$,$Phe_{0.9}$	Pro

[a] Mobilities are given relative to the distance between ε-N_2ph-Lys and aspartic acid.

TABLE VIII: Peptides Isolated from Peptide T_6 after Digestion with Pepsin.

Peptide	Mobility[a]	Composition	NH_2-Terminal
T_6Pa	0.97	$CMCys_{0.9}$,$Asp_{1.0}$,$Gly_{1.0}$,$Leu_{1.0}$, $Tyr_{0.9}$	Asp
T_6Pb	N	$CMCys_{0.8}$,$Lys_{0.8}$,$His_{0.6}$,$Asp_{0.9}$,$Ser_{2.8}$,$Glu_{1.0}$,$Pro_{2.0}$,$Gly_{1.8}$,$Ala_{0.8}$,$Val_{1.8}$,$Leu_{1.4}$	Ser
T_6Pc	1.06	$CMCys_{1.4}$,$Asp_{0.9}$,$Gly_{1.0}$,$Leu_{1.0}$	Asp
T_6Pd	N	$Ser_{2.9}$,$Val_{2.0}$,$Leu_{0.6}$	Ser
T_6Pe	N	$Lys_{0.8}$,$His_{0.7}$,$CMCys_{0.8}$,$Asp_{1.1}$,$Glu_{0.9}$,$Pro_{2.3}$,$Gly_{2.0}$,$Ala_{1.0}$	Pro

[a] Mobilities are given relative to the distance between ε-N_2ph-Lys and aspartic acid.

TABLE IX: Peptides Isolated from Light Chain after Digestion with Pepsin and Trypsin.

Peptide	Mobility[a]	Composition	Sequence
P-T_1	0	$CMCys_{0.3}$,$Thr_{1.3}$,$Ile_{1.1}$,$Arg_{1.0}$	Ile-Thr-Cys-Arg
P-T_2	0.41	$CMCys_{0.4}$,$Ser_{0.7}$,$Glu_{2.1}$,$Tyr_{1.0}$	Tyr-Cys-Gln-Gln-Ser
P-T_3	0.52	$CMCys_{0.4}$,$Val_{1.1}$,$Leu_{1.0}$	Val-Cys-Leu
P-T_4	0.75	$CMCys_{0.2}$,$Glu_{1.1}$,$Ala_{1.0}$,$Tyr_{0.9}$	Tyr-Ala-Cys-Glu

[a] Mobilities are given relative to the distance between aspartic acid and ε-N_2ph-lysine.

residue of the CH_3 region intrachain disulfide bridge. This peptide was not found in the present study. T_7 is bound to a peptic peptide, Thr-Cys-Thr-Leu, which may be related to TPb and peptide T_1.

Comparative studies with an α heavy-chain disease protein (unpublished results) indicated that T_7 and the TPb peptide were present. Hence, they and presumably the longer peptide T_1 probably came from the Fc region and may represent the CH_2 homology region intrachain bridges. Peptide T_8, whose function and location are unknown, is not present in a heavy-chain disease protein and consequently, probably is located in the Fd region.

The α chain appears unusual since it contains several additional cysteine residues not found in any of the other classes of Ig. In addition to the bridges characteristic of each domain, the H–L, the previously recognized C-terminal cysteine, and at least one inter-H-chain bridge in the hinge, there appear to be several extra cysteines not present in other immunoglobulin heavy chains. One is an intrachain bridge between the hinge and the T_2 peptide which is probably located in the Fd fragment. The other involves the hinge and the T_6 peptide in the Fc fragment. The detailed structure and a possible model for these peptides bound to the hinge are outlined in the accompanying paper (Wolfenstein-Todel *et al.*, 1973). It should be noted that the structural arrangement of several cysteines in the Fc fragment, among them the two remaining cysteine residues from peptide T_6 as well as the carboxy-terminal cysteine of the α chain, remain uncertain. It seems possible that some of them may be involved in bridging to the secretory piece or the J chain. An additional point of interest is that the interchain bridges of IgA appear to be less labile than those of the other classes of Ig since all but the carboxy-terminal cysteines were labeled when the molecule was completely reduced and radioactively alkylated after having been partially reduced and alkylated with cold iodoacetic acid.

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