Immunoglobulin A. Arrangement of Disulfide Bridges in the "Hinge" Region of an Immunoglobulin A1 Human Myeloma Protein†

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ABSTRACT: The arrangement of disulfide bridges in the hinge region of an IgA1(κ) myeloma protein (Oso) was studied by diagonal electrophoresis of a subtilisin digest prepared from a fraction isolated after peptic–tryptic digestion of the protein. The cysteic acid containing peptides were isolated and their sequence was established. Subtilisin split the hinge region in two main peptides; one came from the N-terminal end, contained one cysteine residue, and was bound by an intrachain bridge to another peptide probably in the Fd fragment. The second, a pentapeptide, containing two cysteines, was bound to another peptide in the Fc fragment also containing two cysteines. Several possible models for the hinge are discussed. The existence of two intrachain disulfide bridges involving the hinge region is unique for the IgA heavy chain and suggests an unusual degree of folding of the α chain.

In a previous study (Frangione and Wolfenstein-Todel, 1972) we reported the amino acid sequence of the "hinge" region of an IgA1[1] myeloma protein (Oso). A 30-residue peptide was isolated, which contained 3 cysteines, 12 prolines and carbohydrate. Its sequence showed some unusual features, since it contained at least two identical stretches of seven or eight residues. Comparison with the sequence of the "hinge" peptide derived from an IgA2 protein (Wolfenstein-Todel *et al.*, 1972) showed that they were identical in the first eight residues and in the last five, but that the hinge region of IgA2 lacked carbohydrate and had a gap of about 12–13 residues just in the place where the duplication of a small fragment was shown in IgA1. Thus, it seems possible that the hinge region of IgA1 molecules is the result of the insertion of partially duplicated gene segments in the immunoglobulin genes. The sequence is

Val-Thr-Val-Pro-Cys-Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Pro-Ser-Thr-Pro-Pro-Thr-Pro(Ser-Pro-Ser)Cys-Cys-His-Pro-Arg
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Peptic–tryptic diagonal maps of an IgA2 myeloma protein (Wolfenstein-Todel *et al.*, 1972) failed to provide a model for the cysteine residues in the hinge region, since it was not possible to split the hinge peptide with the different enzymes used and because two other peptides were always found in the same region of the diagonal map as the hinge.

In this report, we present results obtained by diagonal electrophoresis after subtilisin digestion of the 30-residue hinge region of an IgA1 protein (Oso). The findings permit some tentative conclusions as to the arrangement of the cysteine residues and point out once again the unique structural features of the hinge of the α1 chain.

Material and Methods

Myeloma protein Oso was purified as described for protein Pat (Wolfenstein-Todel *et al.*, 1971). Immunological and chemical typing (Frangione and Franklin, 1972) showed it to be IgA1(κ). Procedures for enzyme digestion, column chromatography, partial reduction and radioactive alkylation, purification of peptides, amino acid analysis, and determination of amino acid sequences were all as described in the previous paper (Frangione and Wolfenstein-Todel, 1972). Cysteine diagonal maps were carried out at pH 3.5 by the method of Brown and Hartley (1966).

Results

A peptic–tryptic digest of myeloma protein Oso was fractionated on a column of Sephadex G-50, as previously reported (Frangione and Wolfenstein-Todel, 1972). Material eluted under the first peak (see Figure 1, Frangione and Wolfenstein-Todel, 1972) was partially reduced and alkylated with ^{14}C-labeled iodoacetic acid and the radioactive peptides were purified by paper electrophoresis. Table I shows their amino acid composition, N-terminal amino acid residues, and electrophoretic mobilities at pH 6.5. They are related to peptides T3, T6, and T2 (Mendez *et al.*, 1973), respectively. Peptide PT1 runs as a series of radioactive bands with mobilities between 0.1 and 0.3, probably due to its high content of carbohydrate. Peptide PT2 was submitted to the dansyl-Edman procedure and showed the sequence

Ala-Val-Glx-Gly-Pro-Pro-Glx-Arg-Asp-
Leu-Cys-Gly-Cys-Tyr-Ser-Val-Ser

which is in agreement with the one previously reported (Wolfenstein *et al.*, 1971) for a peptide that included the last nine amino acids. The reason why trypsin did not split the bond Arg–Asp in this experiment is not clear. The sequence of peptide PT3 was established by the dansyl-Edman procedure as follows

Pro-Ala-Thr-Gln-Cys-Leu-Ala-Gly-Ser-Lys

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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).

TABLE I: Amino Acid Composition of Peptic–Tryptic Carboxymethylcysteine Peptides Obtained from Peak 1 of a Peptic–Tryptic Digest of Protein Oso (IgA1).

Peptide	PT1	PT2	PT3
Lys			1.0
His	0.9		
Arg	1.0	1.0	
CM-Cys	2.2	1.4	0.7
Asp		1.2	
Thr	4.2		0.9
Ser	5.1	2.3	1.2
Glu		1.9	1.0
Pro	11.5	2.0	1.1
Gly		2.3	1.2
Ala		1.0	2.4
Val	2.5	1.7	
Leu		1.0	1.0
Tyr		0.7	
CHO	+	+	
Mobility at pH 6.5	0.1–0.3	0.46	0
N-Terminal	Val	Ala	Pro

TABLE II: Amino Acid Composition of Cysteic Acid Peptides Derived by Subtilisin Digestion from Peak 1 of a Peptic–Tryptic Digest of Protein Oso (IgA1).

Peptide	PTS 1a	PTS 1b	PTS 2a	PTS 2b	PTS 2c	PTS 2d
His					1.0	1.1
Arg					0.9	1.0
CyA	1.0	1.1	2.1	1.1	2.1	1.0
Asp			1.0	0.9		
Thr		4.6				
Ser		4.7				
Pro		10.8			1.0	0.9
Gly			1.1	1.0		
Ala	1.0					
Val		2.8				
Leu	1.0		1.1	1.0		
CHO		+				
Mobility at pH 6.5	0.67	0.10	1.2	0.95	0.11	−0.12
N-Terminal	CyA	Val	Asp	Asp	CyA	CyA
Yield (μM)	0.02	0.02	0.25	0.01	0.20	0.01

This sequence is the same as the one of peptide T2 (Mendez *et al.*, 1973).

Another fraction of peak I was further digested with subtilisin, and the cysteic acid peptides were identified on a diagonal map at pH 3.5 (Figure 1). Two main sets of peptides were isolated and purified by high-voltage electrophoresis at pH 6.5 and 2.1. Their amino acid composition, N-terminal residues, and electrophoretic mobilities are shown in Table II. Further characterization of the peptides was as follows.

Peptide PTS 1a. The sequence of this tripeptide was established by the dansyl-Edman procedure, and is

Cys-Leu-Ala

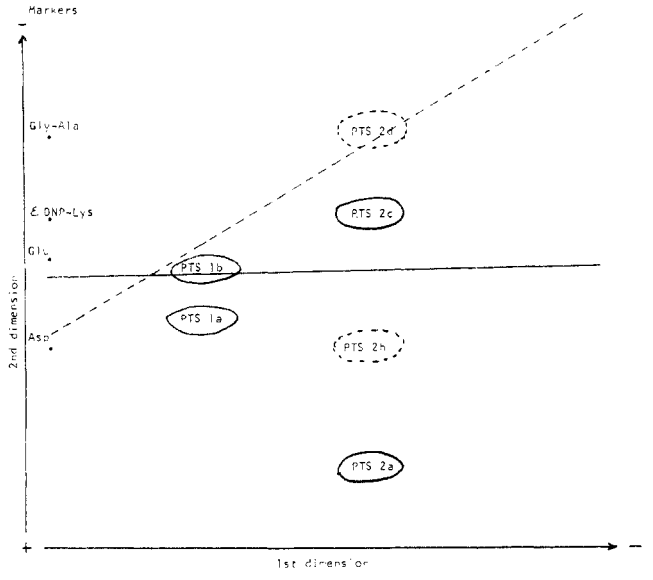

FIGURE 1: Diagram of the diagonal electrophoresis of a subtilisin digest of peak 1 obtained after peptic–tryptic digestion of protein Oso (IgA1). Electrophoresis in both dimensions was at pH 3.5. The amino acid composition of the peptides is shown in Table II; PTS = pepsin–trypsin–subtilisin.

Peptide PTS 1b. It corresponds to the amino terminal of the hinge as was previously described (Frangione and Wolfenstein-Todel, 1972). This peptide contains carbohydrate and only a single cysteine residue (at position 5). The dansyl-Edman procedure yielded the following sequence

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)

Peptide PTS 2a. The sequence of this acidic peptide was established by the dansyl-Edman method as follows

Asp-Leu-Cys-Gly-Cys

Peptide PTS 2c. Its sequence was

Cys-Cys-His-Pro-Arg

It represents residues 26–30 of the hinge and contains the last two cysteines. After each step of the Edman degradation this peptide was submitted to paper electrophoresis at pH 6.5, and the changes in mobility as well as the staining characteristics corroborate its sequence.

Peptide PTS 2b (Asp-Leu-Cys-Gly) which contains the first of the Cys residues of PTS 2a, and PTS 2d (Cys-His-Pro-Arg) which contains the last Cys of the hinge were obtained in a low yield, and represent fragments of PTS 2a and PTS 2c, respectively. Peptide PTS 2d results from the cleavage by subtilisin of the peptide bond between two cysteines which is an unusual event, though not unique since it was also observed by Schlesinger *et al.* (1972) in neurophysin.

Since they run together in the first dimension on electrophoresis, and only separate after oxidation in the second dimension, it can be concluded that peptides PTS 1a and 1b are bound to each other by a disulfide bridge. Peptide PTS 1a (Cys-Leu-Ala) is derived from another peptide Pro-Ala-Thr-Gln-Cys-Leu-Ala-Gly-Ser-Lys (PT3, Table I) so that the sequence of this disulfide bridge is as follows

Val-Thr-Val-Pro-Cys-Pro-Val-Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser- · · ·
|
Pro-Ala-Thr-Gln-Cys-Leu-Ala-Gly-Ser-Lys

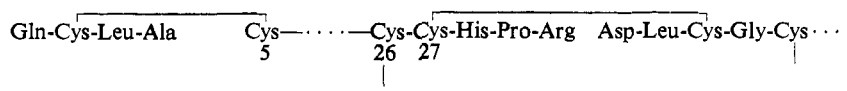

FIGURE 2: Proposed arrangement of disulfide bridges in the hinge region of IgA1.

Peptides PTS 2a and c are also bound to each other, as are 2b and d, and based on their different yields, we conclude that the cysteines of 2b and d are involved in a disulfide bridge whose most likely arrangement is

Cys-Cys-His-Pro-Arg
|
Asp-Leu-Cys-Gly-Cys

Peptides PTS 2a and 2b are derived from another peptide (PT2, Table 1) with the sequence

Ala-Val-Glx-Gly-Pro-Pro-Glx-Arg-Asp-
Leu-Cys-Gly-Cys-Tyr-Ser-Val-Ser

Peptide PTS 2c (Cys-Cys-His-Pro-Arg) was also found in low yield in a more basic section of the diagonal map, either with another low yield peptide related to 2a, or by itself.

Discussion

The purpose of this study was to establish the structural relations of the cysteine residues in the hinge region of the α1 chain. Diagonal electrophoresis at pH 3.5 of a subtilisin digest of the tryptic–peptic peak containing the hinge region gave good resolution since subtilisin splits the hinge before the last two cysteine residues (26 and 27). Isolation and characterization of these peptides and their partners allowed unequivocal assignment of the disulfide bridge involving the first cysteine residue of the hinge peptide (5) and the peptide Cys-Leu-Ala. This peptide was not present in an α-chain disease protein (unpublished observation) and consequently probably comes from the Fd fragment of the α chain. It is probably joined to the hinge region through an intrachain bond because we were unable to detect it after partial reduction and alkylation. It appears likely that the last cysteine of the hinge region is linked to residue 3, of the peptide Asp-Leu-Cys-Gly-Cys. This peptide was found in an α-heavy-chain disease protein, thus placing it in the Fc fragment. Because both peptides contain two cysteines, several different models can be postulated for this region of the molecule. While the precise arrangement cannot be stated with certainty we have chosen the ones shown below on the basis of finding the smaller peptides PTS 2b and 2d, each with only a single cysteine residue in the same region of the diagonal map.

(1) Two symmetric interchain bridges and 1 intrachain bridge per α chain

---Cys-Cys-His-Pro-Arg Asp-Leu-Cys-Gly-Cys
---Cys-Cys-His-Pro-Arg Asp-Leu-Cys-Gly-Cys

(2) Two labile intrachain bridges per α chain

---Cys-Cys-His-Pro-Arg Asp-Leu-Cys-Gly-Cys

(3) Four asymmetrical interchain bridges

⟶Cys-Cys-His-Pro-Arg Asp-Leu-Cys-Gly-Cys⟶
⟵Cys-Gly-Cys-Leu-Asp Arg-Pro-His-Cys-Cys⟵

The results of diagonal electrophoresis do not allow us to differentiate among these possibilities. However, the finding by Abel and Grey (1971) of a hinge peptide containing three cysteines both as a dimer and a monomer together with our finding that the first cysteine is involved in an intrachain bridge indicate the presence of at least one heavy–heavy chain disulfide bridge, thus eliminating model 2 and leaving models 1 and 3. As indicated by the arrows in the diagram, model 3 would require both α chains to be antiparallel. Although it cannot be disproved at the present time, this is highly unlikely since detailed studies of a γ3 myeloma protein suggested that at least one and possibly all subclasses of γ chains are parallel (Frangione and Milstein, 1968).

Hence, IgA1 contains at least one inter-heavy disulfide bridge in the hinge region and perhaps at least one additional one in the fifth residue of the Asp-Leu-Cys-Gly-Cys peptide although alternatively this could be involved in an intersubunit disulfide bridge. A model for this region of the molecule is shown in Figure 2.

When the same approach was used on the hinge peptide of IgA2, it proved to be extremely resistant to proteolysis. Two peptides were always found in the same region of the diagonal map as the hinge. They were: Asp-Leu-Cys-Gly-Cys-Tyr-Ser and Glx-Cys-Pro-Asp-Gly-Lys (Wolfenstein-Todel *et al.*, 1972). Owing to the fact that the C-terminal sequence of the hinge of α1 and α2 chains is the same, it seems probable that the structure proposed for IgA1 applies also to IgA2 and that the first peptide is bound to the last cysteine of the hinge. The other peptide (Glx-Cys-Pro-Asp-Gly-Lys) has homologies to the peptide (Glu-Cys-Leu-Ala-Gly-Ser-Lys) which is bound to the first cysteine of the hinge of the α1 chain since the substitution of Leu by Pro and Ala by Asp can be explained by a single nucleotide change in the codon. Hence it seems likely that it too forms an intrachain disulfide bridge with the corresponding cysteine in the hinge of the α2 chain and that the arrangement of the disulfide bridges of the two hinges is similar in spite of their remarkable structural differences.

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