STRUCTURAL FEATURES OF THE PORCINE IMMUNOGLOBULIN-G INTERCHAIN DISULFIDE BRIDGES.

J.J. Metzger and P. Pery

Service d'Immunologie, Station de Virologie et d'Immunologie,
Institut National de la Recherche Agronomique
78850 THIVERVAL-GRIGNON (France)

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SUMMARY :

The interchain disulfide bridges were studied on the porcine immunoglobulin-G molecule by radioalkylating the labile half-cystine residues after reduction in physiological buffer. The distribution of the radioactivity was followed on the isolated heavy and light chain and on the Fab and Fc fragment peptide maps. This distribution was not uniform according to the subclasses. The peptides were isolated. The sequence of one of the heavy chain peptides bearing the light chain was determined and proved to be similar to corresponding regions in the immunoglobulin-G molecules of other mammalian species.

INTRODUCTION :

Immunoglobulin-G molecules are seric proteins having antibody activity. They have a four polypeptide chain, disulfide linked structure (5). The diversity of their antibody specificity is related to differences in the amino-acid sequences of the variable regions (5). Another level of heterogeneity is found in the constant region of the molecule according to the subclasses. This heterogeneity gives differences in the number and positioning of the interchain disulfide bridges and is reflected in the biological properties of the molecule (8). This former property has been used as an easy method of subclass identification (9).

In the pig, immunoglobulin classes have been described and are well characterized (7,3). The subclass identification, however, is dependent upon the availability of specific antisera (10) because of the absence of monoclonal myelomas or antibodies of restricted heterogeneity in this animal. The aim of the present study is to add further to the knowledge of the interchain disulfide bridges in the porcine immunoglobulin-G molecule (12).

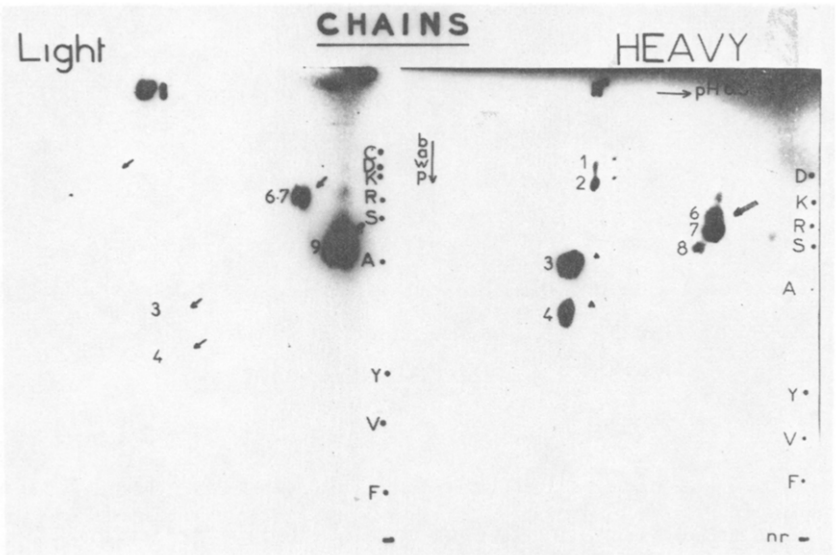

Fig. 1 : Radioautography of the finger-prints of the labelled Ig G chains. 2 mg of light chain trypsic peptides and 3 mg of heavy chain trypsic peptides were submitted to the pH 6.5 electrophoresis then to the butanol, acetic acid, water, pyridine (15;3;12;10) descending chromatography. Autoradiographies were made with Kodirex, exposure time 2 to 8 days. Marker spots are the standard amino acids. Radioactive peptides are numbered as indicated.

MATERIAL AND METHODS :

The isolation and labelling of Ig G molecules have already been described (10) : the protein was dissolved in Tris-HCl, 0.2 M buffer, pH 8.0 and subsequently reduced for 2 hours under nitrogen by 5 mM dithiothreitol. Radioalkylation was performed in the dark, using [^{14}C] -iodoacetic acid (Duphar, Holland) 0.10 mC per gram of Ig G. The pH was maintained constant at 8.0 by the addition of NaOH 0.5 N.

Partial hydrolysis by trypsin gave rise to the Fab and Fc fragments. The 50 000 M.W. peak fragments (10) were purified by ion-exchange chromatography over DEAE-Sephadex A-25 equilibrated with Tris-HCl 0.01 M buffer pH 7.6, containing 0.05 M NaCl. The Fab_t fragment was not retained under such conditions. The pure Fc_t fragment was eluted from the column when the NaCl molarity reached 0.15 M. Their purity was checked by immunoelectrophoresis. Complete reduction and alkylation of the Fab_t fragment rendered the Fd_t fragment insoluble in 5 M urea and allowed a partial purification of this fragment.

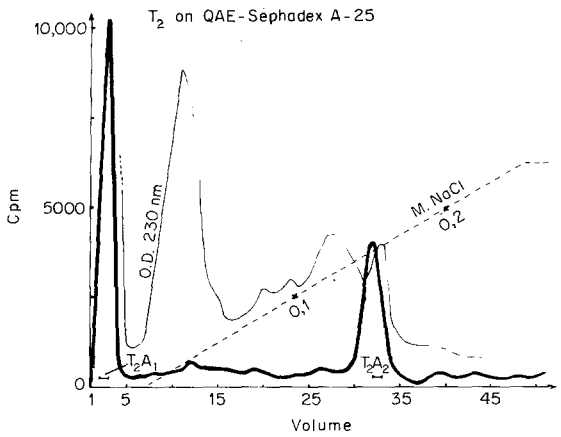

Fig. 2 : QAE-Sephadex purification of the radioactive peptides. T_1 and T_2 are the radioactive peaks from a Sephadex G-25 chromatography. These peaks were put on a QAE-Sephadex equilibrated in 8 M urea, 0.01 M Na-acetate buffer pH 5.5. The heavy line represents elution of the [^{14}C] radioactivity, the light line, the optical density at 278 nm or at 230 nm. The T_2A_1 peptides were further purified by Dowex 1x2 chromatography.

Following complete reduction by 0.2 M 2-mercaptoethanol and alkylation with 0.3 M iodoacetic acid in 6 M guanidine-HCl, Tris-HCl 0.2 M buffer pH 8.0, heavy and light chains were isolated by Sephadex G-100 chromatography using 5 M urea in 0.05 M formic acid (12).

The preparation of the peptide maps, their radioautography and the measurement of radioactivity of the peptides have already been described (11).

Radioactive peptides were purified from a fully reduced and alkylated, labelled Ig G which was cleaved by a 3 hour contact with trypsin, 1/50 (w/w) in 0.05 M ammonium carbonate pH 8.5 and then chromatographed on Sephadex G-25.

The resulting radioactive peaks T_1 and T_2 were submitted to a Q A E-Sephadex A-25 chromatography equilibrated in a Na-acetate 0.01 M buffer pH 5.5, containing 8 M urea. The elution of the radioactive peptides was performed using a O.O to 0.3 M NaCl molarity gradient. Further purification

Footnote : The nomenclature of the immunoglobulins and their fragments follows the W.H.O. recommendations (19). The amino acid sequences are indicated using the one-letter notation system (I.U.P.A.C. - IUB - CBN - 1968).

peptide n°	H	L	Fab_t	Fc_t	Fd_t
1	570				
2	850				
3	3800	415	210	55	755
4	2750	250	280	50	560
5				560	
6	3020	590	20	1980	8
7			10	440	10
8				320	
9	160	2660	80	160	88

Table 1 : Radioactivity of the disulfide bearing peptides from the Ig G chains and fragments. The radioactive areas were cut out and counted in toluene phase on a Packard liquid scintillation spectrometer. Results are given in net Cpm. Numbering is the same as in Fig. 1.

was achieved by means of Dowex 1x2 chromatography in pyridine-acetate 0.2 M with a 6.0 to 5.0 pH gradient and of paper chromatography. The determination of the amino-acid sequence was performed by the Edman substractive method up to the eleventh residue and by recurrent carboxypeptidase hydrolysis for the Gly-Arg C-terminus (1, 17). Subtilisin and mild acidic hydrolysis allowed overlapping radioactive peptides. The cysteinyl-residue was determined as Dansyl-C.M.cystein and by the radioactivity of the thiazolinone derivative.

RESULTS AND DISCUSSION :

I - Recognition of the interchain disulfide bridges on the peptide maps.

The distribution of the radioactive peptides of heavy and light chains is shown in Fig. 1 and in Table 1. 18% of the labelling is associated with the light chain. Radioactive peptides from the heavy chain can be divided into those present in the Fab_t map (n° 3 & 4) and those present in the Fc_t map (n° 6, 7 & 8). The light chain peptide n° 9 is present in the Fab_t map and is different from the "Fc_t" peptides (n° 6 & 7) seen as contaminants of the light chain preparation. This contamination is very frequent in the porcine Ig G and is due to a relative lability of the "hinge" region of the heavy chain. All of the peptides found on the fragment maps were retrieved on the chain maps. Thus, the peptides 3 and 4 are heavy chain peptides involved in the heavy-light bond, the peptide n° 9 being the corresponding light chain pep-

		I	II	III
	2	820	1260	1400
"Fd"	3	5170	6820	9000
"Fd"	4	4230	2400	2400
"Fc"	6	3350	4660	7200
"Fc"	7	3220	1765	
"L"	9	1100	940	1130

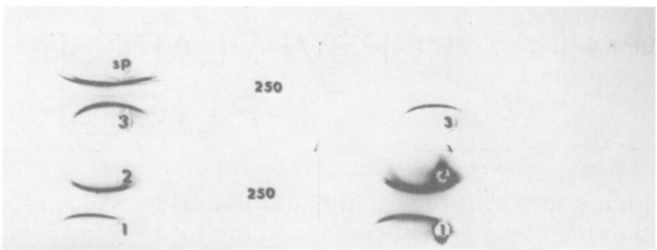

Table 2 : Radioactivity of the disulfide bearing peptides from the different subclass Ig G preparations. Notations are the same as in Table 1. In the immunoelectrophoresis and the radioimmunoelectrophoresis, "I" represents the pure Ig G_2, "II" is the Ig G_2 with some Ig G_1, "III" is Ig G_1 with Ig G_2, "sp" is porcine serum and "250" is the rabbit serum anti-porcine serum which recognizes Ig G_1 from Ig G_2 (10).

tide. The heterogeneous group of peptides n° 6, 7 and 8 includes the inter-heavy chain bridges in the Fc fragment.

II - Distribution of the peptides according to the subclasses.

The separation of the subclasses by classical methods after the radio-alkylation of the total pool of Ig G proved to be difficult. The reason being that the iodoacetic radioalkylation introduced negative charges onto the molecule. Therefore, the subclass separation must be completed before radio-labelling. The peptide maps were prepared from the whole Ig G molecule (Table 2). The resolution of the "Fc" peptides was poor and did not yield demonstrable differences between the subclasses. The main difference resided in the "Fd" peptides : the peptide n° 4 is almost exclusively asso-

		121 130
PORCINE IgG		TAPSVYPLAPCGR
MOUSE	IgG_{2a}	TAPSVYPLAPVCG
	IgG_{2b}	TPPSVYPLAPGCG
RABBIT IgG		KAPSVFPLAPCCG
GOAT IgG		TPPKVYPLTSCCG
GUINEA-PIG IgG_2		TAPSVFPLAASCV
HUMAN	IgG_1	KGPSVFPLAPSSK
	IgG_{2-4}	KGPSVFPLAPCSR
	Ig D	PIISGCR

Table 3 : Homologies in the heavy-light chain disulfide bridge regions. The porcine Ig G peptide n° 3 was sequenced according to the Edman-substractive method (⟶) or the carboxypeptidase method (⟵). The mouse sequences were taken from (16), rabbit from (4), goat from (18), guinea-pig from (2), human Ig G_1 from (6), Ig G_{2-4} from (8, 13) and Ig D from (14). The non-identical residues are underlined.

ciated with the slow Ig G fractions. This result suggests a greater subclass heterogeneity than that previously described (10).

III - Purification of the peptides.

The radioactive peptides were purified by Sephadex G-25 chromatography, then by QAE-Sephadex ion exchange (Fig. 2). A complete separation was obtained : the T_1A_1 peak contained the "Fc" peptides, the T_2A_2 peak corresponded to the light chain peptides, the T_2A_1 peptides, further purified by Dowex 1x2 ion-exchange were the "Fd" peptides which were submitted to a descending paper chromatography.

These two peptides, which differ according to the subclass, do not belong to the same heavy chain part because they had incompatible amino acid composition and they did not derive one from the other after an oxydation. They thus represent different attachment sites of the light chain onto the heavy chains.

The amino acid sequence of the peptide n° 3 was determined. This result and the comparison between homologous regions are given in Table 3. The sequence corresponds to the "120-132" residues in the heavy chain. The outstanding similarity between these amino acid sequences in this region must correlate with a flat spatial configuration which perhaps has some importance in the heavy-light chain recognition in the Ig G molecule (15) but not necessarely identical for the other classes.

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