HETEROGENEITY OF PIG IMMUNOGLOBULIN γ-CHAINS

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1. Introduction

γ-Chains represent heavy chains of the immunoglobulin G class. Heterogeneity of γ-chains has been studied in detail in human species (reviewed in [1]). There are two main regions of amino acid sequence variability. (1) The *N*-terminal region (about 110 amino acid residues) characterized by multiple amino acid replacements. (2) The 'hinge' region which is localized in the middle of the γ-chain. This contains all or most of half-cystines which constitute the inter-chain disulfide bridges of the intact immunoglobulin molecule. The variations in the number and position of inter-chain disulfide bridges correlate with antigenically defined immunoglobulin G subclasses.

The existence of subclasses of pig immunoglobulin G has been postulated in order to explain heterogeneity of immunoglobulin fractions in antigenic properties [2–4], slight differences in peptide maps [2–5], differences in sensitivity to papain splitting and in some biological properties [5]. The aim of this work was to fractionate and to characterize the population of isolated γ-chains, i.e. to examine the heterogeneity of γ-chains under conditions where the heterogeneity of light chains does not interfere.

2. Material and methods

Pig immunoglobulin γ-chains were prepared from mildly *S*-sulfonated immunoglobulin G by gel filtration on Sephadex G-100 in 0.05 M formic acid with 5 M urea [6]. By mild *S*-sulfonation the inter-chain bridge forming half-cystines were converted to *S*-sulfocysteine residues. Urea was an ion-free preparation. 2-^{14}C-Iodoacetic acid was a product of Radiochemical Centre Amersham, England.

Determination of amino acid composition was described before [7]. High-voltage paper electrophoresis at pH 5.6 and preparation of peptide maps were described in previous papers [8, 9].

For radiolabeling of *S*-sulfocysteines 5 mg of a γ-chain preparation were dissolved in 1.2 ml of 0.1 M tris-HCl buffer pH 8.0 containing 6 M urea and the solution was immediately subjected to gel filtration on a Sephadex G-25 column equilibrated with the tris buffer without urea. To 3.2 ml of clear solution of γ-chains obtained in this way, β-mercaptoethanol was added up to 0.1 M concentration and the reaction mixture was kept under nitrogen 1 hr at room temperature. The reaction was then applied to a Sephadex G-25 column (1.2 × 17 cm) equilibrated with nitrogen saturated 0.1 M tris-HCl buffer pH 8.0 containing 4 mM β-mercaptoethanol and 1 mM EDTA. The protein fraction (3.5 ml) emerging from the column was collected under nitrogen and supplemented by 0.7 ml 0.02 M radioactive iodoacetic acid (10.5 mCi/mmole). After 30 min the radioactively alkylated γ-chains were transferred into a 0.2% ammonium carbonate solution pH 8.5 by gel filtration on a Sephadex G-25 column. An aliquot was taken for determination of carboxymethylcysteine content. The temperature of the remaining sample solution was raised to 37° and trypsin was added at an enzyme to substrate ratio 1:100. After two hours of digestion thermolysin was added at the same weight ratio and the sample

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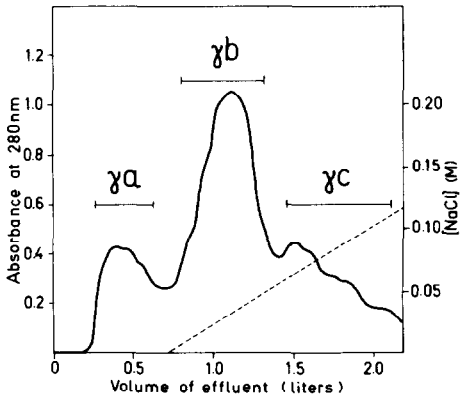

Fig. 1. Ion-exchange chromatography of pig immunoglobulin γ-chains on DEAE-Sephadex A-50. The column (3.8 × 58 cm) was equilibrated with 0.02 M sodium phosphate buffer pH 6.7 containing 8 M urea. 800 mg of the sample dissolved in 25 ml of buffer was applied to the column. A linear gradient of electrolyte concentration was developed by mixing 1.5 liters of the initial buffer in the mixer with 1.5 liters of the buffer containing 0.3 M sodium chloride in the reservoir. —, absorbance at 280 nm, - - - molarity of sodium chloride in the effluent.

was kept at 37° for two hours more. The digest was freeze-dried and applied to a strip of Whatman 3 MM paper. After performing an electrophoretic run at pH 5.6 the distribution of radioactivity was evaluated by scanning in a Frieseke-Hoepfner FH 452 counter.

Table 1
Amino acid composition of γ-chain fractions. Numbers of residues were normalized to a total of 450 amino acid residues in the γ-chain.

Amino acid	Number of residues of fraction:			D[a]
	γa	γb	γc	%
Lysine	24.6	23.4	21.8	12.2
Histidine	8.6	8.1	8.3	3.6
Arginine	17.7	15.8	16.5	7.1
Half-cystine[b]	12.7	13.5	14.4	12.5
Aspartic acid	33.0	32.6	32.8	0.6
Threonine	37.4	37.9	37.8	1.1
Serine	44.8	48.6	47.7	6.2
Glutamic acid	41.7	43.3	43.9	5.1
Proline	38.6	37.0	37.7	2.4
Glycine	36.1	34.4	34.1	5.7
Alanine	24.5	25.5	25.6	4.4
Valine	43.9	43.2	42.8	2.5
Methionine	4.5	4.2	4.2	6.9
Isoleucine	12.2	12.5	12.8	4.8
Leucine	28.8	28.3	28.0	2.8
Tyrosine	16.8	17.4	17.1	1.8
Phenylalanine	14.7	14.5	14.6	0.7
Tryptophan[c]	9.8	9.5	9.9	1.0
Total	450.0	450.0	450.0	

[a] Absolute value of the residue number difference between the most basic (γa) and most acidic fraction (γc) expressed in percent, i.e. 200.|γa−γc|/(γa+γc).
[b] Determined as cysteic acid.
[c] Determined spectrophotometrically.

3. Results

γ-Chains were resolved by ion-exchange chromatography on DEAE-Sephadex into three fractions designated γa, γb, and γc (fig. 1). The fraction γc was free of γb contamination by re-chromatography. The amino acid composition of individual γ-chain fractions displayed only minute differences. Differences in the content of lysine and half-cystine were relatively most pronounced (table 1). The weight balance showed that the content of non-protein moiety in γ-chain fractions was less than 10%.

The majority of peptide spots in maps of tryptic digests was common to all three γ-chain fractions (fig. 2). Very few spots were characteristic for a single fraction.

The selective alkylation of half-cystines by radioactive iodoacetic acid was performed after replacing the *S*-sulfo groups by sulfhydryl groups under mild conditions. In the alkylated γ-chain fractions 4.8 to 5.0 residues of carboxymethylcysteine per a total of 450 residues were found by amino acid analysis. The electrophoretic pattern of the radioactive peptides was characterized by three main peaks (fig. 3). The radioactivity in the main peaks was identical within the limits of experimental error. The pattern of all three fractions contained several minor radioactive peaks. Only one peak was significantly distinctive (see fig. 3).

4. Discussion

The population of normal pig immunoglobulin γ-chains exhibited an almost continuous spectrum

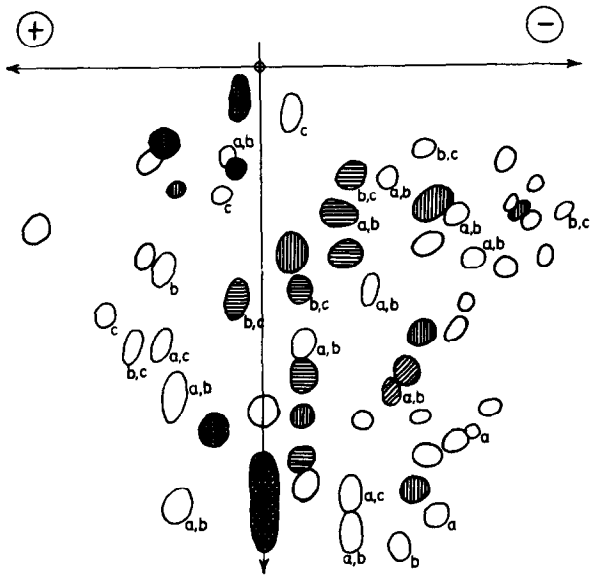

Fig. 2. Composite peptide map of tryptic hydrolyzates of γ-chain fractions. In horizontal direction electrophoresis in pH 5.6 buffer containing pyridine–acetic acid–water (4:1:245, by vol.), in vertical direction chromatography in butanol–pyridine–acetic acid–water (15:10:3:12, by vol.). Origin is at the top in the middle. Peptides were visualized by ninhydrin and additional specific detections. Horizontal hatching: tyrosine- and histidine-containing peptides. Vertical hatching: arginine-containing peptides. Slanted hatching: tryptophan-containing peptides. Peptides common to all samples compared (i.e. γa, γb, and γc) are shown without lettering. Occurrence of peptides characteristic for one or two samples is indicated by lettering.

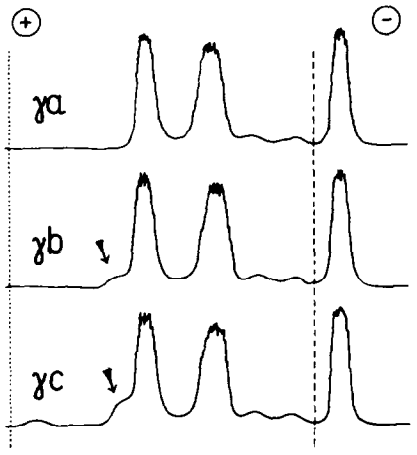

Fig. 3. Scanner records of the radioactivity in the electropherograms of enzymic digests of ^{14}C-carboxymethylated γ-chain fractions. Electrophoresis was performed at pH 5.6. Verical dashed line indicates the origin. Verical dotted line at the left side inidicates the position of glutamic acid marker. The disitnctive peak of radioactivity is marked with an arrow.

of components upon ion-exchange chromatography. This fact prevented us from using the procedures elaborated for immunoglobulin light chains [8] and isolating γ-chain subpopulations homogeneous with respect to electric charge. Thus, three broad fractions were cut out of the whole spectrum of γ-chain components and their chemical characteristics were compared. It was assumed that possible heterogeneity in the number of *S*-sulfocysteine residues would cause a distribution of the components upon ion-exchange chromatography. This assumption was at least partly confirmed by the finding of an increasing content of half-cystine residues in successive fractions (see table 1). At the same time the decreasing content of lysine residues indicated that other amino acids, irrespective of the region of their origin, also took part in the electric charge heterogeneity.

We expected that an examination of peptides containing radiolabeled half-cystines [10] could contribute significantly to the elucidation of subclass composition of normal pig immunoglobulin, particularly if peptides from isolated heavy chains were examined. The finding of identical patterns of main radioactive peptides in all three γ-chain fractions indicated that the major subpopulation of pig γ-chains were uniform with respect to the number and position of inter-chain disulfide bridges. It is likely that this uniform major subpopulation of pig γ-chains has the character of a subclass. The finding of a minor distinctive radioactive peptide and of the different content of half-cystine residues in individual γ-chain fractions are consistent with previous indications [2–5] that pig immunoglobulin G consists of more than one subclass.

Analytical data prompt some conclusions on the number of inter-chain disulfide bridges in the major subclass of pig γ-chains. The mean number of half-cystine residues in pig γ-chains is 14 . Eight half-cystines can be assumed to form the inter-chain disulfide loops which occur generally in immunoglobulin heavy chains. One half-cystine forms the linkage be-

tween the heavy and the light chain. Thus the data obtained in the present study indicate that the major subclass of pig immunoglobulin G possesses five inter-heavy chain disulfide bridges.

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