N- AND C-TERMINAL SEQUENCES IN PIG IMMUNOGLOBULIN π-CHAINS: ASSIGNMENT TO THE K TYPE

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1. Introduction

π-chains, representing one of the structural types of pig immunoglobulin light chains were prepared for the first time by Franěk and Zorina [1]. Their characteristics such as the N-terminal amino acid and the amino acid composition of the C-terminal peptide, did not indicate any relationship to the κ-chains of human immunoglobulin. Therefore, the tentative conclusion was made that, in addition to the λ-chains, pig immunoglobulin contains a new structural type of light chains, i.e., the π-chains. Independently, Hood and co-workers [2] determined the sequence of two peptides from the tryptic hydrolyzate of pig immunoglobulin light chains and designated them as the C-terminal peptides of λ-chains and κ-like chains. In the structural study reported in the present communication the data on the amino acid sequence at the N- and C-termini of the π-chains were extended and a significant relationship to the κ-chains of human and mouse immunoglobulins was revealed.

2. Methods

The preparation of immunologically pure pig immunoglobulin, oxidative sulphitolysis, separation of polypeptide chains by gel filtration, ion exchange chromatography of the light chains on SE-Sephadex, preparation of tryptic hydrolysates, preparation of peptide maps and determination of N-terminal amino acids by dinitrophenylation were described in detail before [1].

The peptides were purified by high voltage paper electrophoresis at pH 1.9 or 5.6 [3,4] and by descending chromatography [4]. Aminoethylation of the cysteine residues in peptides was carried out according to Raftery and Cole [5] in 0.021 M ammonium carbonate. The aminoethylated peptide was separated from the reaction mixture on a column of Sephadex G-10 equilibrated with 0.021 M ammonium carbonate and further purified by high-voltage electrophoresis at pH 1.9.

The N-terminal amino acid residues were determined by dansylation [6]. The dansyl amino acids were identified by thin-layer chromatography using silica gel "Silufol" (product of the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague) [4,7].

Edman degradation was usually employed together with the dansyl technique [8]. The PTH-derivatives of aspargine and of glutamic acid were identified by chromatography on silica gel thin layers in the E and F systems of Edman and Sjöquist [9].

3. Results and discussion

As a starting material for the study of terminal sequences, the rechromatographed π-chains were used. By comparing the peptide map of this preparation with the peptide maps of other light chain types, it could be confirmed that the π-chain preparation was free of admixtures of other types. The only N-terminal

Abbreviations:
PTH: phenylthiohydantoinyl
Dansyl: 1-dimethylaminonaphthalene-5-sulfonyl

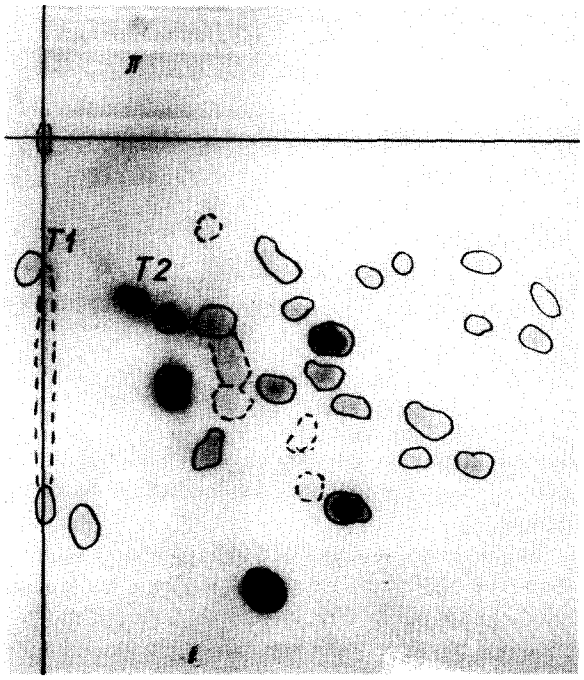

Fig. 1. Peptide map of S-sulfonated π-chains. Electrophoresis was carried out at pH 1.9. The origin is at the top left corner. Horizontally electrophoresis, vertically chromatography (*n*-butanol : pyridine : acetic acid : water 15 : 10 : 3 : 12). Anode at the right.

amino acid found was alanine, at an amount of 0.7 mole per mole of protein.

The symbol T 1 on the peptide map (fig. 1) stands for the previously identified C-terminal pentapeptide [1,2]. From the tryptic hydrolysate of the S-sulfo π-chains, subjected to high-voltage electrophoresis at pH 1.9 and to descending chromatography, both the above mentioned peptide T 1 and another acid peptide, designated T 2, were obtained. The amino acid composition of the two peptides is shown in table 1. The amino acid sequence determined after performic acid oxidation of the peptides by Edman degradation and by dansylation, is shown in fig. 2. The T 2 peptide is evidently the C-terminal nonapeptide, a result of the slow cleavage of the Arg-Asn bond by trypsin. Subsequent tryptic hydrolysis splits it into a tetrapeptide Ser, Phe, Asn, Arg (peptide T 2–T-1 in fig. 2) and a peptide identical with T 1.

Table 1
Amino acid composition of peptides T 1 and T 2. The numbers represent molar ratios, uncorrected for losses during hydrolysis. Cysteine was determined as cysteic acid in oxidized peptides.

	T 1	T 2
Cys	0.97	1.00
Arg	–	0.94
Asp	1.03	1.91
Ser	–	0.87
Glu	2.06	2.03
Ala	1.05	1.01
Phe	–	1.00

The T 1 peptide contains three and the T 2 peptide four acid residues. Therefore, additional information on the possible presence of amide groups was necessary. The N-terminal asparagine and the adjacent glutamic acid of the T 1 peptide were determined after Edman degradation as the corresponding PTH-derivatives. The T 2–T-1 peptide (see fig. 2) behaves as a

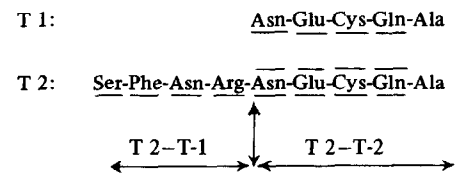

Fig. 2. Determination of amino acid sequence of peptides T 1 and T 2. —— Sequence established by Edman degradation. The amino acid sequence of the fragment T 2–T-2 was determined in separate experiments, independent of the determination of the sequence of the whole peptide T 2.

basic substance on electrophoresis at pH 5.6. Consequently, the residue of aspartic acid is present in the amide form. The aminoethylated T 1 peptide behaves on electrophoresis at pH 5.6 as neutral, the penultimate C-terminal glutamic acid residue is thus present in the amide form.

The observed C-terminal sequence of pig π-chains resembles greatly the C-terminal sequence of mouse κ-chains (table 2). In all the K type chains known so far the C-terminus has the sequence Ser-Phe-Asn-Arg-X-X-Cys. The fact that this sequence was also found near the C-terminus of the π-chains strongly suggests that π-chains belong to the K type. The C-terminal dipeptide Gln-Ala of pig π-chains has obviously no significant function. One can easily imagine that its

Table 2
Comparison of terminal amino acid sequences in κ-like light chains.
Residues that are invariant in all three species are printed in italics.
X stands for unidentified amino acid at the given position.

Species	Amino acid sequence	References
Pig	H-Ala-*Ile*-Ala-$^{Val}_{X}$-$^{Leu}_{X}$-*Thr-Glx*-	this paper
	H-Ala H-Glu-Ile or Leu-	[2]
Mouse, protein 70 and 46	H-Asp-*Ile-Val-Leu-Thr-Gln*	[14, 17]
Man, subclass III	H-Glu-*Ile-Val-Leu-Thr-Gln*-	[2, 10, 11, 13, 18, 19]
Pig	-*Ser-Phe-Asn-Arg*-Asn-*Glu-Cys*-Gln-Ala-OH -Asx-*Glx-Cys*-Glx-Ala-OH	this paper [2]
Mouse	-*Ser-Phe-Asn-Arg*-Asn-*Glu-Cys*-OH	[14, 15]
Man	-*Ser-Phe-Asn-Arg*-Gly-*Glu-Cys*-OH	[10, 12, 16]

deletion did not decrease the affinity of the light chains for the heavy chains and thus did not markedly affect the formation of antibodies.

In order to obtain further structural data for supporting or rejecting the classification of the π-chains we subjected intact π-chains to Edman degradation combined with dansyl technique (table 2). In some positions (3 and 4 from the N-terminus) the identification of dansyl amino acids on a thin layer revealed more residues than one but most of them could not be unequivocally determined. The amino acid sequence in table 2 thus represents only the major variants; besides these, there exist π-chains with other, so far unknown residues in the positions shown. The main N-terminal sequence closely resembles the N-terminal sequence of human Bence-Jones proteins of Putnam's subclass "Glu" [10], subclasses II and III of Milstein [11] and Hilschmann [12] or κ_{Smi} of Niall and Edman [13], or finally of the SK II subclass of human and mouse Bence-Jones proteins of Hood and coworkers [2].

All the results presented here indicate that the pig π-chains thus represent one of the possible evolutionary stages of immunoglobulin κ-chains.

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