SEQUENCE OF *N*-TERMINAL 46 AMINO ACID RESIDUES IN PIG IMMUNOGLOBULIN λ-CHAINS

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1. Introduction

It is assumed that the diversity of antibody specificities is generated by multiple amino acid replacements which have been found in the *N*-terminal portions of immunoglobulin polypeptide chains. In previous papers [1, 2] we described the isolation of a fragment of the *N*-terminal variable part of pig immunoglobulin λ-chains. The fragment, which was named T2, consisted of two peptides joined together by a disulfide bond. The amino acid sequence of one of these peptides (T2b) containing 22 residues was determined and several amino acid replacements were found [3]. The other peptide (T2a) contained the *N*-terminus of the λ-chains; the amino acid composition indicated that it contained 47 amino acid residues. The peptide T2a could be split into two peptides (T2a1 and T2a2) by tryptic hydrolysis after aminoethylation of the half-cystine residue [2]. The determination of the primary structure was complicated by the fact that the *N*-terminal group of the λ-chains was blocked. Significant progress was made by using mass spectrometry. This method revealed a sequence of six amino acid residues from the *N*-terminus and confirmed that the *N*-terminal amino acid was pyrrolidone carboxylic acid [4]. A complete amino acid sequence of peptide T2a is reported in this paper.

2. Materials and methods

The starting material was the peptide T2a and its components T2a1 and T2a2, the isolation of which from the tryptic hydrolyzate of the λ-chains of pig immunoglobulin was described in detail before [2].

Determination of the amino acid sequence of small peptides by stepwise degradation combined with dansylation and determination of the *N*-terminal amino acids by dansylation was described in detail in a preceding paper [3]. Alternatively, Edman degradation was carried out according to Blombäck and coworkers [5] and the PTH*-amino acids were identified by thin-layer chromatography [6].

Peptides were hydrolyzed by thermolysin in 0.2% ammonium carbonate for 6 hr at 37°. Cyanogen bromide cleavage of the peptides was performed in 70% formic acid [7].

Peptides of the thermolysin digests and peptides formed by cyanogen bromide cleavage were purified by high-voltage paper electrophoresis at pH 1.9 or 5.6, and by descending paper chromatography. Details of these methods as well as of the detection techniques were described previously [3, 8, 9]. Determination of the amino acid composition of the peptides was also described before [2]. Homoserine lactone was converted to homoserine before determination of amino acids [10].

3. Results and discussion

Peptide T2a was hydrolyzed by thermolysin and those of the resulting peptides which yielded intense and distinct zones when separated by paper techniques were isolated, characterized and sequenced (peptides

* *Abbreviation:* PTH = phenylthiohydantoinyl.

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Table 1
Amino acid composition of peptides.
The source of individual peptides is indicated in tables 2 and 3. The numbers represent moles of amino acid residues per mole of peptide.

Amino acid	Peptide T2a1 [a]	T2a2 [a]	T2at1	T2at2	T2at3	T2at4	T2a1b1	T2a1b2	T2a1b1t1	T2a1b1t2	T2a1b1t3	T2a1b2t1	T2a1b2t2	T2a1b2t3
Arginine	–	1.0	–	–	–	1.0	–	–	–	–	–	–	–	–
AE-Cysteine [b]	1.0	–	–	1.0	–	–	–	0.9	–	–	–	–	–	0.9
Aspartic acid	0.2	1.4	–	–	–	–	–	–	–	–	–	–	–	–
Threonine	3.7	2.7	–	1.0	–	1.0	1.0	2.9	1.0	–	–	1.0	1.0	1.0
Serine	2.5	4.5	1.0	–	3.0	–	–	2.0	–	–	–	1.1	–	–
Glutamic acid	3.1	3.1	–	–	–	3.0	3.0	–	1.0	2.0	–	–	–	–
Proline	1.8	4.1	–	–	–	3.1	1.1	0.9	–	1.0	–	1.0	–	–
Glycine	2.2	2.7	–	–	1.1	1.0	–	2.0	–	–	–	1.9	–	–
Alanine	1.1	1.1	–	1.0	–	–	1.0	–	–	–	1.0	–	–	–
Valine	2.8	1.2	–	–	–	–	1.0	2.0	–	0.9	–	1.0	1.0	–
Methionine	0.9	–	1.0	–	–	–	1.0[c]	–	–	–	1.0[c]	–	–	–
Isoleucine	0.8	–	–	–	–	–	0.9	–	–	0.9	–	–	–	–
Leucine	1.6	–	–	1.0	–	–	–	1.1	–	–	–	–	–	1.0
Tyrosine	–	1.2	–	–	–	–	–	–	–	–	–	–	–	–
Phenylalanine	0.2	1.8	–	–	1.0	0.9	–	–	–	–	–	–	–	–
Tryptophan	–	0.9	–	–	–	–	–	–	–	–	–	–	–	–
Total	21.9	25.7	2.0	4.0	5.1	10.0	9.0	11.8	2.0	4.8	2.0	6.0	2.0	2.9

[a] Taken from reference [2]; the amount of *S*-aminoethylcysteine or arginine was used as a basis for calculation of the composition.
[b] *S*-Aminoethylcysteine.
[c] Determined as homoserine.

Table 2

Amino acid sequence of peptide T2a1.

Amino acids found as minor variants are set below the main sequence. Arrows indicate residues determined by sequential degradation. Unusual abbreviations: Glp = pyrrolidone carboxylic acid, Hsr = homoserine, Aec = *S*-aminoethylcysteine.

Final sequence	Glp–Thr–Val–Ile–Gln–Glu–Pro–Ala–Met–Ser–Val–Ser–Pro–Gly–Gly–Thr–Val–Thr–Leu–Thr-Cys
	Ser Val
	Pro
Mass spectrometry [a]	Glp–Thr–Val–Ile[a]–Gln–Glu
	Ser Val
	Pro
T2aT1[b]	Met–Ser
T2at2[b]	Leu–Thr–Aec–Ala
T2a1b1[c]	(Glx, Thr, Val, Ile, Glx, Glx, Pro, Ala, Hsr)
T2a1b2[c]	Ser-Val–Ser–Pro-Gly (Gly, Thr, Val, Thr, Leu, Thr, Aec)
T2a1b1t1[d]	(Glx, Thr)
T2a1b1t2[d]	Val (Ile, Glx, Glx, Pro)
T2a1b1t3[d]	Ala–Hsr
T2a1b2t1[e]	Val–Ser–Pro–Gly–Gly–Thr
T2a1b2t2[e]	Val–Thr
T2a1b2t3[e]	Leu(Thr, Aec)

[a] Taken from ref. [4]; mass spectrometry does not distinguish between isoleucine and leucine.
[b] Peptides isolated from the thermolysin hydrolyzate of the whole peptide T2a.
[c] Products of the cyanogen bromide cleavage of peptide T2a1.
[d] Peptides isolated from the thermolysin hydrolyzate of peptide T2a1b1.
[e] Peptides isolated from the thermolysin hydrolyzate of peptide T2a1b2.

T2at1, T2at2, T2at3 and T2at4 in tables 1 to 3). The amino acid balance showed that these peptides do not represent the entire section investigated.

Peptide T2a1 which contains one methionine residue (table 1) was split by cyanogen bromide. Paper electrophoresis at pH 5.6 showed that the cleavage products contain, in addition to the remainder of unsplit peptide T2a1, two peptides: one basic, the other acidic, ninhydrin-negative. This last peptide was visualized by chlorine detection. The amino acid composition of the acidic (T2a1b1) and basic (T2a1b2) peptide shows that the two peptides together form the peptide T2a1 (table 1). Peptide T2a1b1 contains homoserine and no detectable *N*-terminal amino acid. Sequential degradation of peptide T2a1b2 yielded a sequence of five residues (table 2). Peptides T2a1b1 and T2a1b2 were split by thermolysin and the resulting peptides were isolated by paper techniques. Information on the amino acid sequence obtained with the aid of these peptides is summarized in table 2. Using mass spectrometry data, the complete sequence of peptide T2a1 could be obtained. According to amino acid analysis a total of 22 residues were expected [1, 2]; the sequence shows that the peptide T2a1 contains 21 residues.

The amino acid sequence of peptide T2a2 was almost entirely determined by sequential degradation. Peptide T2at4 served for completing the *C*-terminal

Table 3

Amino acid sequence of peptide T2a2.

Amino acids found as minor variants are set below the main sequence. Arrows indicate residues determined by sequential degradation.

Final sequence	Ala	Phe	Ser	Ser	Gly	Ser	Val	Thr	Thr	Ser	Asn	Tyr	Pro	Gly	Trp	Phe	Gln	Gln	Thr	Pro	Gly	Gln	Pro	Arg
											Asn							Gly	Thr					
											Thr							Ser	Asn					
																			Gly					
Sequential degradation of the whole peptide [a]	Ala	Phe	Ser	Ser	Gly	Ser	Val	Thr	Thr	Ser	Asn	Tyr	Pro	Gly	Trp	Phe	Gln	Gln	Thr	Pro	Gly	Gln		
											Asn							Gly	Thr					
											Thr							Ser	Asn					
																			Gly					
T2at3 [b]		Phe	Ser	Ser	Gly	Ser																		
T2at4 [b]																Phe	Glx	Glx	Thr	Pro	Gly	Glx	Pro	Arg

a) Amino acids in individual steps determined as phenylthiohydantoin derivatives.
b) Peptides isolated from the thermolysin hydrolyzate of the whole peptide T2a.

part of the sequence (table 3). In three positions simultaneous occurrence of several amino acids was demonstrated.

Sequence of the peptide T2a1 (21 residues) and of the peptide T2a2 (25 residues) yields information on the structure of the *N*-terminal section of pig immunoglobulin λ-chains covering almost one-half of the variable part. The variable character was significantly demonstrated in five positions. Demonstration of a replacement is difficult if the variant represents only a small fraction of the material so that the result cannot be distinguished from the noise generally accompanying the methods of stepwise degradation. Minor variants may be lost if the longer peptide is fragmented and the resulting shorter peptides are purified. Experience gathered here as well as in preceding work [3, 11] indicates that investigation of variable sequences yields most complete results if stepwise degradation of long peptides or mass spectrometry can be used.

It cannot be excluded that pig λ-chains contain sequence variants differing from the sequence reported here in positions which appeared as invariant in the present study. The starting material for the preparation of fragment T2 was not a complex preparation of λ-chains but only one of three main λ-chain fractions and the yield of the fragment T2 did not fully correspond to the theory [2, 3]. In any case, the major sequence found here represents one of the more significant variants of the *N*-terminal section of pig λ-chains. The sequence provided unique data for interspecies comparisons to which a separate communication will be dedicated.

Peptide T2a was also isolated from a pig antibody to the dinitrophenyl group which was affinity-labelled by a reaction with *m*-nitrobenzenediazonium fluoroborate [12]. Most of the label contained in the λ-chains was found in this peptide. Since there is only one tyrosine in the section corresponding to the peptide T2a (table 3), it is assumed that this tyrosine is the specifically labelled residue which is in some way related to the antibody binding site.

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