TENTATIVE SEQUENCE OF 52 AMINO ACID RESIDUES FROM THE CONSTANT PART OF PIG IMMUNOGLOBULIN κ CHAINS

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1. Introduction

The present knowledge of the primary structure of normal immunoglobulin light chains is merely fragmentary. The pig immunoglobulin light chains of the K type have been characterized by the *C*-terminal sequence of nine amino acids [1]. In our preceding paper [2], the isolation of some fragments from the tryptic digest of normal pig immunoglobulin κ chains and the elucidation of the *N*-terminal sequence of 16 amino acid residues have been reported.

In this paper, the amino acid sequence of two additional fragments isolated from the same digest is described. A comparison of the determined amino acid sequence with those of the human and mouse type K Bence-Jones proteins has shown that the two fragments represent a continuous section of the primary structure of pig κ chains between positions 155 and 206 (numbered according to [3]), the homology of which to the two species of the Bence-Jones proteins amounts to 60% of identical residues.

2. Material and methods

The starting material for the present study was the tryptic digest of pig κ chains. Its preparation and preliminary fractionation on Sephadex G-50 in 0.25 M formic acid and on SE-Sephadex C-25 in 8 M urea at pH 3.0 has been described in detail earlier [2].

The amino acid analysis, the reduction and aminoethylation of the disulfide bond, the digestion of the peptides with thermolysin, and the sequential analysis of peptides by the stepwise degradation combined with dansylation have also been described in the preceding paper [2].

The fractionation of some peptides was effected by ion-exchange chromatography on QAE-Sephadex A-25 (1.2 × 21 cm column) using 0.01 M acetate buffer pH 5.0, 8 M in urea, or on SE-Sephadex C-25 (1.2 × 21 cm column) using 5 mM formate buffer pH 3.0, 8 M in urea. A gradient of ionic strength was produced by the addition of sodium chloride to the acetate buffer (for chromatography on QAE-Sephadex) or by the addition of potassium chloride to the formate buffer (for chromatography on SE-Sephadex). The column effluent was monitored either continously at 254 nm or its absorbance at 280 nm was measured afterwards. The material from the pooled fractions was transferred into 1 M acetic acid on a column of Sephadex G-25 and freeze-dried.

The tryptic digestion of peptides was carried in 0.2% ammonium carbonate 4 hr at 37° at an enzyme-substrate ratio of 1:50 (w/w).

Small peptides were fractionated by paper techniques (by high-voltage electrophoresis at pH 1.9 or 5.6 and by descending chromatography [4]) or by gel filtration on columns of Sephadex G-15 or G-25 (1.25 × 80 cm) equilibrated with 0.2% ammonium carbonate.

The *C*-terminal amino acids of peptides were split

Abbreviations:
Dansyl : 1-dimethylaminonaphthelene-5-sulfonyl
PTH : phenylthiohydantoinyl
AE-Cys: *S*-β-aminoethylcysteine.

off by hydrazinolysis [5] and determined as dansyl derivatives by one-dimensional ascending chromatography on a thin-layer "Silufol" [6].

The Edman degradation of peptides was effected by the slightly modified procedure of Blombäck and coworkers [7]. The PTH-amino acids were identified by thin-layer chromatography on silica gel [8, 9].

The presence of amides in peptides was determined according to their mobility during electrophoresis at pH 5.6 or by the determination of the corresponding PTH-amino acids after Edman degradation.

3. Results and discussion

Fraction T3, which had been obtained from the tryptic digest of the κ chains by chromatography on Sephadex G-50 (cf. fig. 4 in [2]) was fractionated further by chromatography on SE-Sephadex at pH 3.0. The material eluted from the column by 0.07 M potassium chloride in a single peak was then fractionated by chromatography on QAE-Sephadex at pH 5.0. In this way, two fragments were obtained, Q-0.06 and Q-0.14, which showed identical amino acid composition (table 1) but differed in their positions on the chromatogram. The fragments were eluted by 0.06 M and 0.14 M sodium chloride, respectively. The amino acid sequence of fragment Q-0.06 was determined by Edman degradation and individual amino acids were identified as phenyl thiohydantoins. To confirm the determined amino acid sequence, the fragment was digested with thermolysin. The digest was fractionated on the Sephadex G-15 column and by paper electrophoresis and the terminal amino acids of the isolated peptides (table 1) were determined.

Comparison of the peptide maps of the thermolysin digests of fragments Q-0.06 and Q-0.14 showed that fragment Q-0.14 represents the determined form of fragment Q-0.06, the determined residues being glutamines 8 and 19. The weight ratio of fragment Q-0.06 to fragment Q-0.14 was 9:1. The total yield of both forms is 16% of the theoretical.

Another fragment investigated in this study, AE-0.16, had been obtained from fraction S-5 (cf. fig. 5 in [2]), reduced by dithiothreitol and aminoethylated with ethylene imine, by chromatography on SE-Sephadex at pH 3.0. According to its amino acid analysis (table 2), peptide AE-0.16 contains 30 amino acid residues and its yield is 10% of the theoretical. The sequence of the first ten amino acid residues at the *N*-terminus of the fragment was established by stepwise degradation combined with dansylation. To determine the sequence of the remaining amino acid residues, fragment AE-0.16 was digested with trypsin and the

Table 1
Amino acid compositions of the fragments Q-0.06 and Q-0.14 and of the peptides obtained from thermolysin hydrolysate of the fragment Q-0.06
Numbers represent molar ratios

	Q-0.06	Q-0.14	Q-Tl-1	Q-Tl-2	Q-Tl-3
Lys	1.96	1.96			1.00
Asp	3.03	3.18	0.94		1.00
Thr	0.99	1.00			0.90
Ser	4.02	4.10		1.90	1.00
Glu	2.95	3.18		0.98	1.88
Gly	1.98	2.04	0.97	1.02	
Val	3.89	3.90	1.06	1.92	1.00
Ile	1.00	0.87			
Leu	1.07	1.09			
Trp	0.90	0.89			
Total	21.85	22.21	2.97	5.82	6.78

Table 2

Amino acid composition of the fragment AE-0.16 and of the peptides from its tryptic and thermolysin hydrolysates. Numbers represent molar ratios. The number of amino acid residues in the determined sequence (cf. fig. 1) is given under "found".

	AE-0.16	Found	A-T-1*	A-T-2	A-T-3	AT1-Tl-1	AT1-Tl-2	AT1-Tl-3
AECys	1.00	1	0.58	1.00				
Lys	1.00	1			1.00			
His	1.91	2	1.00		0.97			1.00
Asp	2.02	2	1.95			1.00		0.89
Thr	3.79	4	3.13		1.03	1.00	1.02	
Ser	7.94	8	7.06	1.92		0.95	1.92	1.02
Glu	2.14	2	1.25		1.10		1.00	
Pro	1.00	1	1.05				1.02	
Val	1.21	1			1.16			
Leu	4.86	5	5.12				2.05	1.08
Tyr	2.50	3	2.39	0.20			0.84	
Total	29.37	30	23.53	3.12	5.26	2.95	7.85	3.99

* The numbers given in this column represent the amino acid composition of the mixture of peptide A-T-1 and A-T-1a (cf. fig. 1 and text).

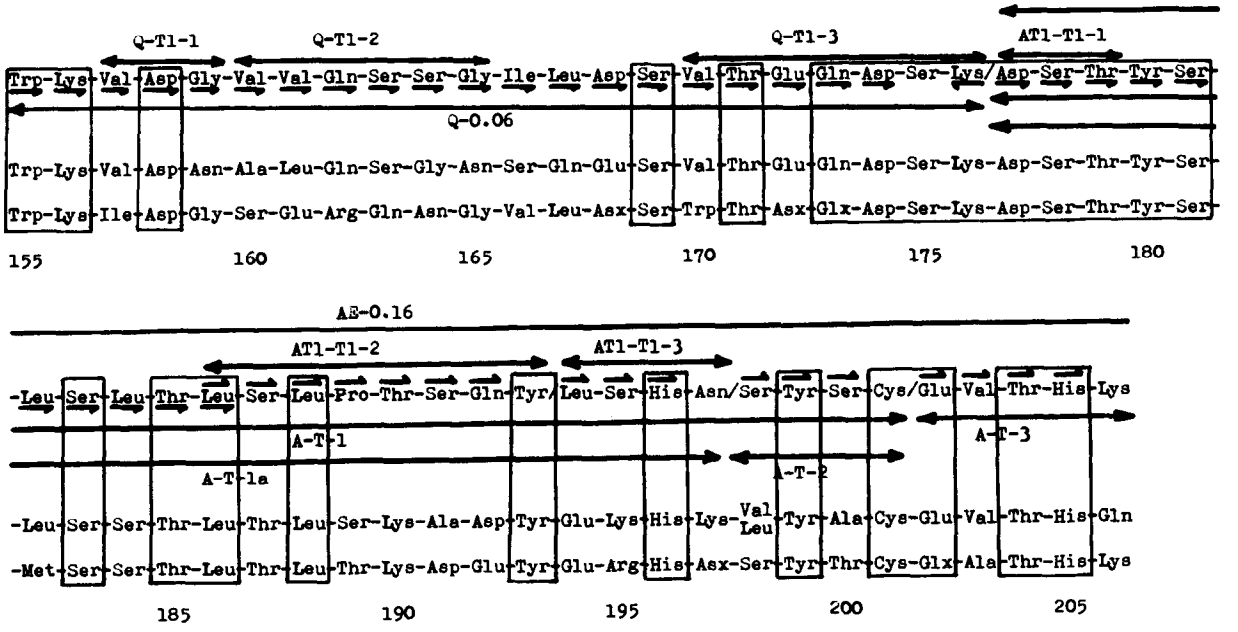

Fig. 1. Comparison of amino acid sequences of fragments Q-0.06 and AE-0.16 with human and mouse Bence-Jones proteins of K type. First line, fragments Q-0.06 and AE-0.16, second line, human Bence-Jones proteins [10], third line, mouse Bence-Jones proteins [11]. The numbering system refers to human Bence-Jones proteins [3]. The arrows designate individual peptides isolated from the tryptic and thermolysin digest (see text). ⇀ Sequence determined by Edman degradation or by stepwise degradation combined with dansylation. ↼ Residue determined by hydrazinolysis. The residues common to all sequences compared are indicated by a frame.

resulting peptides were resolved on a Sephadex G-25 column and by paper electrophoresis. Instead of the two peptides expected (one with *C*-terminal lysine and the other with *C*-terminal amino-ethylcysteine), three peptides (A-T-1 to A-T-3, table 2 and fig. 1) were isolated. Hence, the tryptic digestion was not entirely specific. This non-specific cleavage accounts for the formation of tetrapeptide A-T-2 and thus also for the lower number of serine, tyrosine and aminoethylcysteine residues in the amino acid composition of peptide A-T-1 (table 2). The amino acid sequence of peptides A-T-2 and A-T-3 was determined by stepwise degradation combined with dansylation. Thanks to the high sensitivity of this method, the amino acid sequence of peptide A-T-2 could be determined as Ser–Tyr–Ser–AECys in spite of the fact that most of tyrosine was destroyed during preparation of the peptide (cf. table 2).

Peptide A-T-1 is *N*-terminated with the same amino acid (aspartic acid) as the intact fragment AE-0.16. The fractionation of the thermolysin digest of peptide A-T-1 by paper electrophoresis afforded, among others, also two peptides AT1-Tl-2 and AT1-Tl-3 (cf. table 2 and fig. 1). The determination of their amino acid sequence (by stepwise degradation combined with dansylation) permitted to complete the sequences obtained earlier and thus to derive the amino acid sequence of the whole fragment AE-0.16.

The location of fragment Q-0.06 and of the peptides from fragment AE-0.16 in pig κ chains will emerge from the comparison of the amino acid sequences of the two fragments with the amino acid sequence of human and mouse type K Bence-Jones proteins. Both fragments contain characteristic, infrequently occurring, amino acid residues (tryptophan, half-cystine, tyrosine, histidine) which are invariant in almost all the Bence-Jones proteins. Considering these residues, the fragments can be allocated convincingly to the *C*-terminal half of the pig κ chains, between residues 155 and 206 (numbered according to [3]). The fragments constitute a continuous sequence containing half-cystine 201 (fig. 1). The homology between this sequence and the corresponding sections of the human and mouse Bence-Jones proteins of the K type is 62 and 60% of identical residues. The homology between these sections in human and mouse Bence-Jones proteins is 56%.

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