SELECTIVE ISOLATION OF LARGE HALF-CYSTINE–CONTAINING PEPTIDES

Amino acid sequence near some half-cystines in porcine immunoglobulin γ-chains

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1. Introduction

Diagonal electrophoresis was shown by Brown and Hartley [1] to be the method of choice for determination of the amino acid sequences in the vicinity of cystine residues. Since the method employs paper as supporting medium for peptide separation, it is better suited for isolation of small peptides than of large fragments. We suggest here a complementary method which makes it possible to isolate large half-cystine–containing peptides. The method is particularly useful for sequence studies on microheterogeneous immunoglobulins or on proteins with a very long polypeptide chain, because in these cases a fragmentation of the chain into large pieces is unavoidable [2, 3].

The tryptic digest of an S-sulphonated protein is fractionated by ion-exchange chromatography at pH 3. The fractions obtained are treated with dithiothreitol and then with ethylene imine. Due to the conversion of a negatively charged S-sulphocysteine residue to a positively charged S-(2-aminoethyl) cysteine residue, the half-cystine–containing peptides are recognized by a shift of the elution position upon rechromatography.

2 Materials and methods

Porcine immunoglobulin G with anti-dinitrophenyl specificity was isolated from sera of immune pigs [4]. γ-Chains were separated from the light chains by gel filtration [5]. Urea was an ion-free preparation.

Total S-sulphonation was carried out in guanidine hydrochloride solution at pH 8.6 (0.5 ammonium chloride–ammonia buffer, 6 M guanidine hydrochloride, 5 mM cupric sulphate, 0.2 M sodium sulphite) [6]. γ-Chains (1 g) dissolved in the above medium (150 ml) were allowed to react 1 hr and then transferred to 0.2% ammonium carbonate pH 8.3 on a Sephadex G-25 column for tryptic hydrolysis [7]. Reduction of S-sulphocysteine residues in peptides was done under nitrogen in guanidine hydrochloride solution at pH 8.5 (0.3 M Tris–HCl buffer, 5.5 M guanidine hydrochloride, 0.1 M dithiothreitol). Aminoethylation of reduced peptides was effected by addition of ethylene imine to the above reaction mixture (final concentration, 0.3 M). Modified peptides were desalted on Sephadex G-15 and freeze-dried [3].

The details of other procedures such as resolution of the tryptic digest on Sephadex G-50 [3], ion-exchange separation of large peptides on SE-Sephadex at pH 3 [6, 8], determination of the amino acid composition [3] and stepwise degradation of peptides (manual Edman method) [7] were published before.

3. Results and discussion

The tryptic digest of S-sulpho γ-chains of porcine immunoglobulin (1 g) was first resolved by gel filtration on Sephadex G-50 (fig. 1). The fractions TA and TB were applied separately onto columns of SE-Sephadex and resolved by gradient elution at pH 3.0. The fractions emerging first (TA1 and TB1) are likely to contain most of the half-cystine–containing peptides, because the presence of the S-sulpho group contributes

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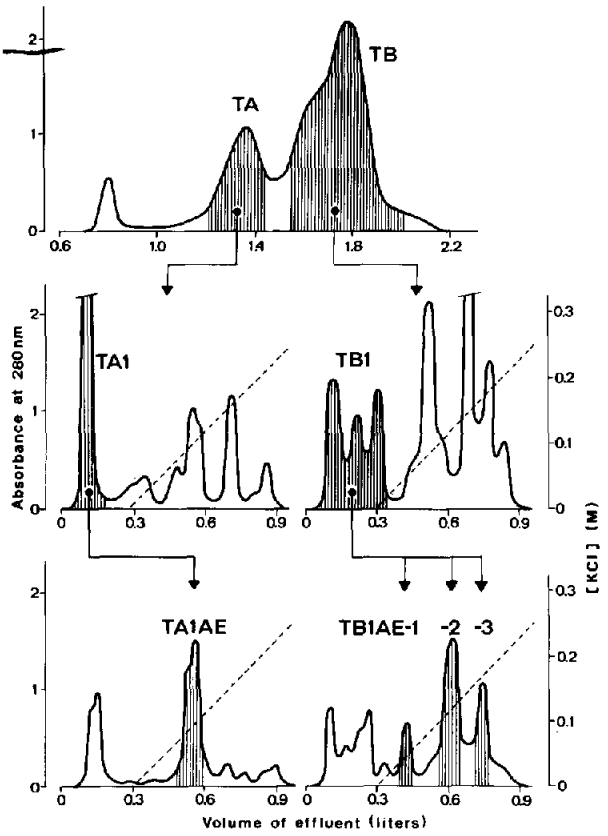

Fig. 1. Isolation of half-cystine–containing peptides from the tryptic digest of porcine immunoglobulin γ-chains. Upper part: Gel filtration of whole tryptic digest on a column of Sephadex G-50 (5.8 × 86 cm) in 0.2% ammonium carbonate pH 8.3; middle part: ion-exchange chromatography of peptide fractions on SE-Sephadex C-25 columns (2.6 × 32 cm) in formate buffer pH 3.0 with urea (5 mM potassium formate, 8 M urea, pH adjusted with formic acid). The ionic strength gradient was produced by additions of potassium chloride to the buffer. The peptides from fractions indicated by hatching were freed of salts and urea by gel filtration on Sephadex G-15 equilibrated with 0.2% formic acid and freeze-dried; lower part: ion-exchange chromatography of aminoethylated peptide fractions on SE-Sephadex C-25. Conditions of separation as above. (——) Absorbance at 280 nm; (—) molarity of potassium chloride in the effluent.

to their electronegativity. All other peptides behave as electroneutral or electropositive, singe β- and γ-carboxyl groups are undissociated at pH 3.0 [3, 6, 8]. Peptide fractions TA1 and TB1 were aminoethylated and rechromatographed under conditions identical with those of the first ion-exchange chromatography (fig.1, lower part).

The material from the double peak TA1AE was repeatedly purified on SE-Sephadex in the pH 3.0 buffer containing 50 mM potassium chloride and yielded a single major homogeneous peptide TA1AE-1 (tables 1 and 2). Peptide TB1AE-1 had serine as the unique N-terminal residue. Its amino acid composition, however, showed that the peptide was not pure. Because of the low yield, this peptide was not studied further. The results of stepwise degradation of whole peptides TB1AE-2 and TB1AE-3 (table 2) were complemented by analyzing peptides resulting from additional tryptic cleavage at the S-(2-aminoethyl) cysteine residues (table 1). The presence of both leucine and isoleucine in the third position of peptide TB1AE-2 was confirmed by the finding of both these amino acids in the pentapeptide TB1AE-2a. The pentapeptide TB1AE-3 was shown to be a mixture of variants having either serine or leucine in the fourth position.

The efficiency of our selective method has been already demonstrated on a relatively uniform protein fragment [8]. The microheterogeneous γ-chain examined in the present work yielded upon tryptic digestion an extraordinarily complex mixture of large and small peptides. Our attention was focused solely on large peptides, the small ones being removed during gel filtration steps. Additional large half-cystine–containing peptides may reside in fractions which have not been examined yet (fig. 1, middle part). Peptides originating in highly variable sections would probably become detectable if a larger amount of starting material is used.

Apart from a small section of sequence from the variable region [7] and a peptide identical with one variant of our peptide TB1AE-3 [9], sequences reported in this communication represent the first structural data from porcine γ-chains. High degree of homology among mammalian immunoglobulins allows the stretches of porcine γ-chain sequence to be aligned with related sequences of other animal species. Peptides TB1AE-3 and TA1AE-1 are according to this alignment adjacent, the respective half-cystines being in positions 142 and 155. Half-cystine in position 142 (peptide TB1AE-3) has been found in human γ2-, γ3-, γ4- and μ-chains [10–13]. It is involved in the heavy–light chain bond. Half-cystine 155 seems to be present invariantly in all species studied so far. It is involved in an intra-chain loop. The sequence of

Table 1
Amino acid composition of half-cystine–containing peptides.

	Moles of amino acid residue per mole of peptide.						
Amino acid	TA1AE-1	TB1AE-2	TB1AE-2a[a)]	TB1AE-2b[a)]	TB1AE-3	TB1AE-3a[a)]	TB1AE-3b[a)]
AE-Cysteine[b)]	1.0	1.0	1.0	–	1.0	1.0	–
Lysine	–	1.0	–	1.0	–	–	–
Arginine	–	–	–	–	1.0	–	1.0
Aspartic acid	2.1	1.9	–	1.9	–	–	–
Threonine	2.8	2.0	1.1	1.1	1.0	1.0	–
Serine	2.9	1.6	0.9	0.9	0.7	0.7	–
Glutamic acid	1.0	1.4	–	1.3	–	–	–
Proline	3.0	2.1	–	2.0	2.9	2.9	–
Glycine	2.0	1.8	–	1.7	1.0	–	1.0
Alanine	2.0	0.7	–	0.5	1.9	2.0	
Valine	3.0	3.4	0.9	2.6	1.0	1.0	–
Isoleucine	–	1.4	0.5	0.9	–	–	–
Leucine	2.0	1.6	0.5	1.2	1.2	1.3	–
Tyrosine	1.0	1.1		1.0	0.9	0.9	–
Phenylalanine	1.0	0.9	–	1.0	–	–	–
Tryptophan	0.8	0.8	–	0.8	–	–	–
Total	24.6	22.7	4.9	17.9	12.5	10.8	2.0

a) Peptides resulting from additional cleavage by trypsin at the S-(2-aminoethyl) cysteine residues. They were isolated by paper electrophoresis and chromatography [8].
b) S-(2-aminoethyl) cysteine.

Table 2
Amino acid sequence of half-cystine–containing peptides.

Peptide	Sequence
TA1AE-1	Asp-Thr-Ser-Gly-Pro-Asn-Val-Ala-Leu-Gly-Aec-Leu-Ala-Ser-Ser-Tyr-Phe(thr_2,glx,pro_2,val_2,trp)
TB1AE-2	Val-Ser-$\frac{Leu}{Ile}$-Thr-Aec-Leu-Val-Thr-Gly-Phe-Tyr-Pro-Pro-Asp-Ile-Asp(lys,ser,$glx_{1.3}$,$gly_{0.7}$,$ala_{0.5}$,$val_{1.5}$,trp)
TB1AE-3	Thr-Ala-Pro-$\frac{Ser}{Leu}$-Val-Tyr-Pro-Leu-Ala-Pro(aec,gly,arg)
TB1AE3b	Gly-Arg
TB1AE-3[a)]	Thr-Ala-Pro-$\frac{Ser}{Leu}$-Val-Tyr-Pro-Leu-Ala-Pro-Aec-Gly-Arg

Aec = S-(2-aminoethyl) cysteine.
a) Complete sequence obtained after incorporation of the sequence of peptide TB1AE-3b.

peptide TB1AE-2 is homologous with a section of the Fc part of human [14] and rabbit [15] γ-chains. The half-cystine of this peptide corresponds to the intra-chain half-cystine in position 390.

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