HUMAN IMMUNOGLOBULIN E. THE PRIMARY STRUCTURE OF THE THIRD CONSTANT ($C_\epsilon 3$) DOMAIN OF THE EPSILON CHAIN

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1. Introduction

The function of immunoglobulin E (IgE) as mediator of allergic reactions of the immediate type is explained by its specific interaction both with a particular antigen (allergen) and with receptors on basophilic leucocytes and mast cells [1]. The cytotropic property of the IgE molecule is a characteristic of the Fc region [2, 3] and in a previous paper the amino acid sequence of the C-terminal domain was reported [4]. In the present paper we report the primary structure of the second last domain ($C_\epsilon 3$) in Fc which appears to be homologous to the $C_\gamma 2$ AND $C_\mu 3$ domains.

2. Materials and methods

IgE myeloma protein was isolated from the serum of patient ND by salting-out (Na_2SO_4) and further purified by ion-exchange chromatography and gel filtration as previously described [1].

For partial reduction–^{14}C–alkylation 2.45 μmoles of lyophilized IgE was dissolved in 0.3 M Tris–HCl–2 mM EDTA Na_2 pH 7.6 (protein concn. 30 mg/ml) and incubated for 30 min at 37°C with N_2-bubbling prior to the addition of 0.3 mmoles of DTT (dithiothreitol, Sigma). After 60 min the reduced protein was alkylated with 0.3 mmoles of ^{14}C-labelled-iodoacetate (specific activity 0.2 mCi/mM) for 45 min at 0°C (icebath) and finally fractionated on Sephadex G-150 (3.2 × 180 cm) in 5% formic acid. Fractions containing epsilon chain were lyophilized (yield 4.6 μmoles) and reduced with DTT (10 mM) in 6 M guanidine HCl–0.3 M Tris–HCl pH 7.6 (protein concentration 10 mg/ml) for 120 min at 37°C.

One portion (A) containing about 2.3 μmoles of the completely reduced epsilon chain was alkylated with 'cold' iodoacetate, another portion (B) was alkylated with ^{14}C-labelled iodoacetate (specific activity 0.2 mCi/mM) for 60 min at 0°C, dialyzed against 1% sodium chloride and 0.05% acetic acid and finally lyophilized. For cyanogen bromide cleavage the lyophilized preparations A and B (about 2.3 μmole each) of completely reduced and alkulated epsilon chain were dissolved in 70% formic acid and cyanogen bromide (Fluka) added to give a CNBr:protein ratio (w/w) of 1:1. After 18 hr at 25°C the two solutions were diluted four times with water and lyophilized. The products were dissolved in 5% formic acid and fractionated on Sephadex G-100 in the same solvent. Six peaks were obtained; fractions under peak III ($V_e/V_t = 0.54$) were pooled concentrated by lyophilization and the product further purified on Sephadex G-50 in 5% formic acid. One major peak ($V_e/V_t = 0.41$) was obtained, which consisted of a single component as revealed by starch gel electrophoresis in acid urea [5]. The fragment (designated A:III and B:III respectively) yielded about 18% of the amount of epsilon chain subjected to CNBr-cleavage. Tryptic cleavage of fragment A:III and B:III was done in 1% NH_4CO_3 for abour 4 hr at 37°C using an enzyme:substrate ratio of 1:100. Chymotryptic cleavage of fragment A:III was done in 1% NH_4CO_3 for about 6 hr at 37°C using an enzyme:substrate ratio of 1:100. Peptic cleavage of fragment B:III was done in 0.01 M HCl for 18 hr at 25°C using an enzyme:substrate ratio of 1:50. The digests (10 mg) were fractionated by paper electrophoresis at pH 6.5 followed by purification of the peptides by paper chromatography in butanol–acetic

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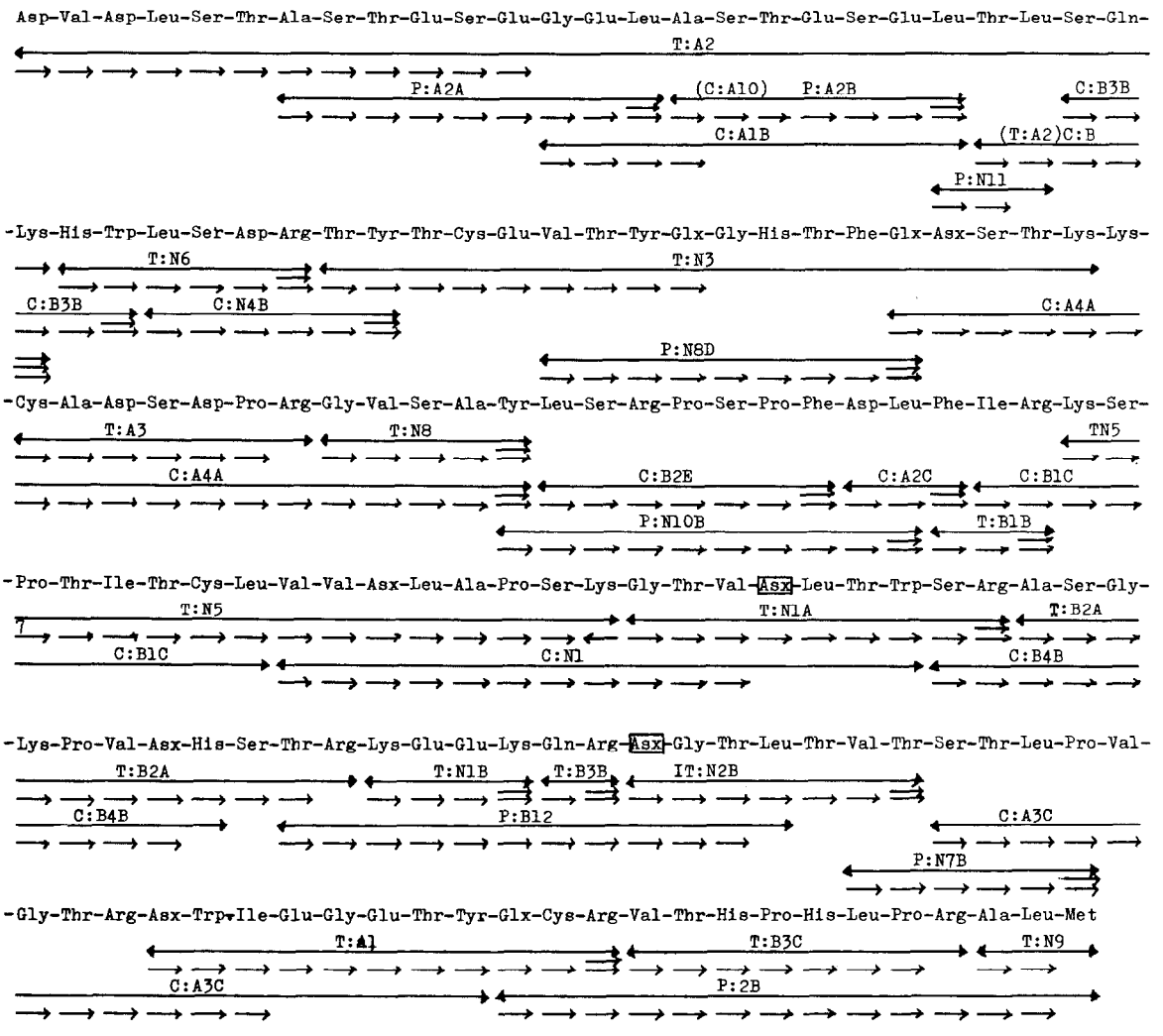

Fig. 1. The amino acid sequence of fragment III. Tryptic(T), chymotryptic(C) and peptic(P) peptides were subjected to the dansyl–Edman procedure as indicated by arrows. Double arrows indicate that the residue was obtained as a free amino acid at the end of the degradation; the C-terminus of TN5 was determined after cleavage with carboxypeptidase B. An Asx within a box indicates the site of attachment of an oligosaccharide side chain.

acid–water–pyridine (15:3:12:10) and paper electrophoresis at pH 3.5. The peptides were analyzed and then subjected to the dansyl–Edman procedure as described previously [6, 7]. Carbohydrate analysis was done by GLC on TMS derivatives of methyl pyranosides as previously described [8]. For selective carboxymethylation of methionyl residues [9] 0.18 μmoles of IgE were dissolved in 4.5 ml of 5% formic acid, alkylated with 168 μmoles of ^{14}C-labelled iodoacetate (specific activity 0.3 mCi/mM) for 50 hr at 37°C. After dialysis against 1% NH_4CO_3 the product was subjected to tryptic cleavage for 5 hr at 37°C

Table 1
Comparison of the carbohydrate composition and amino acid sequence of the glycopeptide regions of C_H3 from human IgE (ND) and IgM (Ou) *

			Residues per molecule				
			Fucose	Mannose	Galactose	*N*-Acetyl glucosamine	*N*-acetyl neuraminic acid
CHO-1 **	$C_\epsilon3$ (ND)	–Gly–Thr–Val–*Asn*–Leu–Thr-Trp–	1	3	2	3	1–2
	$C_\mu3$ (Ou)	–Thr–His–Thr–*Asn*–Ile–Ser–Glu–	1	3	2	4–5	?
CHO-2	$C_\epsilon3$ (ND)	–Lys–Gln–Arg–*Asn*–Gly–Thr–Leu–	–	6	–	2	–
	$C_\mu3$ (Ou)	–Ser–His–Pro–*Asn*–Ala–Thr–Phe–	–	5	–	2	–

* Taken from ref. [11].
** As shown in fig. 3, the side chains are attached to asparaginyl residues at non-homologous positions.

using an enzyme:substrate ratio of 1:100 and the digest fractionated as described above. Radioactive peptides were identified by autoradiography for 24 hr using Kodak RP14 Xomat.

3. Results and discussion

Cyanogen bromide cleavage of completely reduced and alkylated epsilon chain ND yields at least six fragments, of which only two (peak I and peak III), Sephadex G-100) were found to contain [^{14}C]carboxymethylated sulphydryl groups presumably derived from both inter- and intrachain disulphide bonds. Fragment III was selected for the present study since it represents a carbohydrate containing portion of the Fc region (H. Bennich, unpublished results), which might carry the D2-antigenic characteristics of IgE [1] and furthermore be located adjacent to the previously sequenced C-terminal domain of the epsilon chain [4].

Fig. 1. shows the tentative amino acid sequence of fragment III as deduced from the results of dansyl–Edman degradation of overlapping peptides produced by enzymic cleavage.

The amino acid composition derived from the sequence is in close agreement with the composition found by amino acid analysis of a hydrolysate of the intact fragment, except for the values of alanine, glycine and proline, which exceed the expected numbers by one residue each. Although this discrepancy would indicate the possibility that a tri-peptide or a single residue has escaped our attention, the consistent compositions and sequence data of the many individual peptides speak in favour of a correct sequence of fragment III.

Fragment III contains 10 arginyl and 8 lysyl-residues, but only 14 tryptic peptides could be isolated. The sequence contains one –Lys–Lys– and two –Arg–Lys– bonds and tryptic cleavage yielded in addition to free lysine two arginine-peptides (TN5 and TN1B) having an aminoterminal lysyl-residue. In one of the tryptic peptides (TB2A) the presence of both arginine and lysine was explained by the resistance of a Lys–Pro bond to enzymic attack. Two of the tryptic peptides (TN8 and ITN2B) did not contain arginine, lysine or homoserine/homoserine lactone thus indicating the presence of an active 'contaminant' in the trypsin preparation. As shown in fig. 1 the tryptic peptides TA2 and TN3, containing 27 and 18 amino acid residues respectively, were too large for complete degradation by the manual Edman procedure and thus required further cleavage by chymotrypsin and/or pepsin. One tryptic peptide having a prolyl residue in the aminoterminal position and located between TN8 and TN5 (fig. 1) was not isolated.

Concerning the location of fragment III within the epsilon chain, the isolation of a radiolabelled peptide (Ala–Leu–*Met*–Arg) from a tryptic digest of IgE (ND) selectively carboxymethylated at the methionyl-residues favoured the assumption that the carboxyterminus(Met) of fragment III could be linked to the aminoterminus(Arg) of the previously characterized CNBr-fragment $\epsilon4$ [4]. Fragment III contains 4 half-cystinyl residues. After partial reduction, only one of the half-cystines could be [^{14}C]carboxymethylated, which indicates that it participates in the formation of an inter-chain disulphide bridge. After complete reduction, the remaining half-cystines could be radiolabelled; at present we assume that the two half-

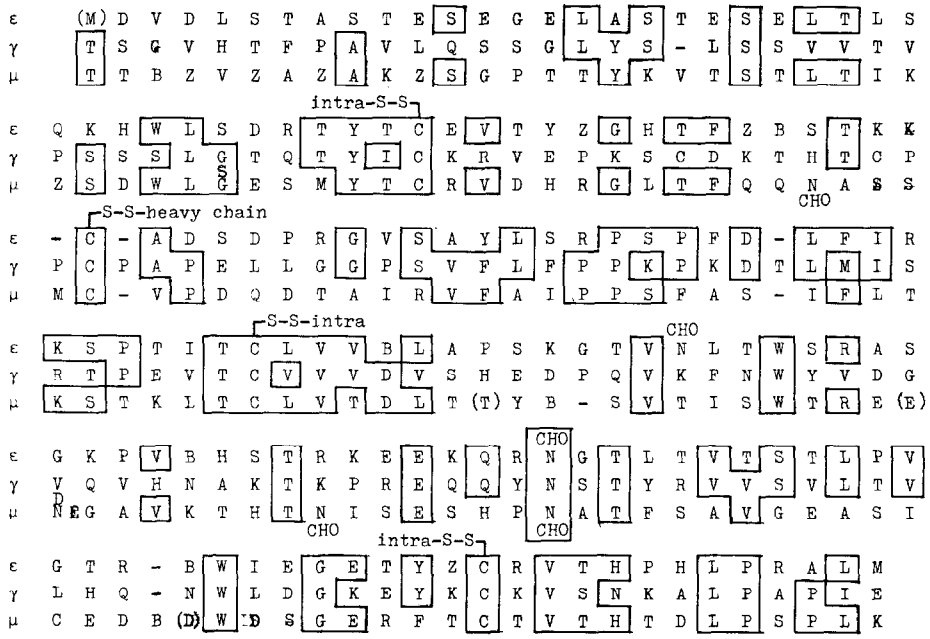

Fig. 2. Comparison of the sequence of fragment III with the sequence of the $C_\gamma 2$-domain [13, 14] and the $C_\mu 3$-domain [11, 16]. The location of an oligosaccharide unit is indicated by CHO. Gaps introduced to maximaze homology are indicated by a horizontal bar.

cystinyl residues located in the carboxyterminal half of fragment III form a disulphide loop within the fragment, while the first half-cystinyl residue in the aminoterminal half participates in a disulphide loop located within the previously postulated extra domain of the epsilon chain [10].

Two oligosaccharide side chains are present in fragment III. Table 1 shows that they differ as regards constituent monosaccharides. Both side chains are located within the postulated disulphide loop of fragment III and presumably attached to the polypeptide chain by a GlcNAc–Asn linkage. The presence of two oligosaccharide units, one having a complex and the other a simple structure, has been described to be a characteristic also of the C_μ-domain [11]. The amino acid sequence of fragment III of the epsilon chain is in many respects very similar to the sequences of the $C_\gamma 2$- and $C_\mu 3$-domains (fig. 2), but the degree of homology is significantly lower than that found for the C-terminal domains of the ϵ-, γ- and μ-chains [4].

The skin-fixing properties of the IgE molecule have been localized to the portion of the epsilon chain comprising the $C_\epsilon 3$- and $C_\epsilon 4$-domains [10], but it is not known at present, whether the binding site(s) is formed by a *cis* or a *trans*-domain interaction or is an intrinsic property of one or the other domain. However, the strikingly high degree of homology between the $C_\epsilon 4$ and $C_\gamma 3$ (fig. 3) might be of particular significance in view of that C_γ seems to be the domain responsible for the cytotropic activity of human IgG [12].

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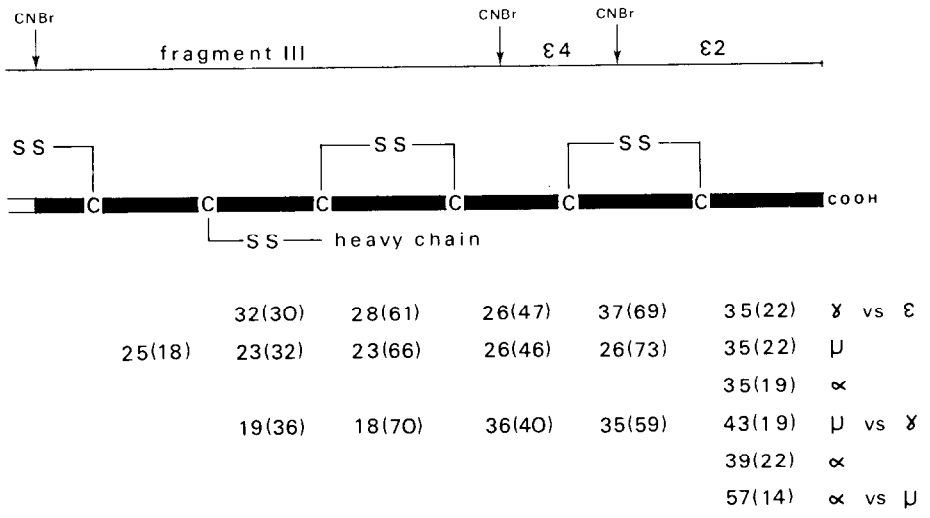

Fig. 3. Comparison of analogous domains and inter-domain regions of the epsilon (fig. 2 and ref. [4]), gamma [13, 14], mu [11, 16] and alpha [15] polypeptide chains. Figures indicate the degree of homology (in per cent and, within brackets, the minimum number of base changes required to convert one sequence into the other; gaps are computed as two base changes.

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