HUMAN IMMUNOGLOBULIN E. THE PRIMARY STRUCTURE OF THE C-TERMINAL DOMAIN OF THE EPSILON CHAIN

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1. Introduction

Immunoglobulin E (IgE), a minor class of human serum proteins [1], plays an important role as mediator in various hypersensitivity reactions of the immediate type [2]. IgE binds firmly to membrane receptors on basophilic leucocytes and mast cells via its Fc-portion [3,4]. A study on the structure of this region of the IgE molecule is therefore of interest and in the present paper a comparison is made between the amino acid sequences of the C-terminal domains of ϵ-, γ- and μ- chains.

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 [2].

For cyanogen bromide cleavage 5 μmoles of lyophilized IgE was dissolved in 70% formic acid and CNBr added to give a CNBr:protein ratio (w/w) of 1:2. After 12 hr at room temp. the solution was diluted four times with water and lyophilized. The product was redissolved in 1 M HAc–25 mM NaCl and fractionated on Sephadex G-100 in the same solvent. Only two peaks were obtained; the first, major peak eluted with the void volume and the second peak with a V_e/VT of 0.61. The latter peak, which yields about 12% of the applied amount, was reduced with dithiothreitol (20 mM) in 6 M guanidine-HCl–0.1 M Tris-HCl pH 8 for 60 min at room temp., alkylated with [^{14}C]iodoacetate, specific activity 1.35 mCi/mM, and the product fractionated on Sephadex G-50 in 1 M acetic acid–25 mM NaCl. Two new fragments were obtained and designated ϵ2 and ϵ4. Tryptic cleavage of the fragments was done in 1% NH_4HCO_3 for 8 hr at 37°C using an enzyme:substrate ratio of 1:100. Peptic cleavage of the fragments was done in 0.02 N HCl for 14 hr at 37°C using an enzyme:substrate ratio of 1:50.

The digests (1 μmole) were fractionated by paper electrophoresis at pH 6.5 followed by purification of peptides by paper chromatography in butanol–acetic 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 [5,6].

Automatic amino-terminal sequence determination [7] was carried out on a Beckman 890 Sequencer, using Beckman chemicals. 800 nmoles of ϵ2 was subjected to fifty cycles of degradation with the standard double-cleavage programme. ϵ4 (1 μmole) was degraded using a single-cleavage programme designed to reduce extractive losses: the Quadrol buffer was diluted to 0.25 M with propan-2-ol:water (3:2, v/v), the coupling time increased to 30 min and the ethyl acetate wash reduced to 400 sec. The use of thiol

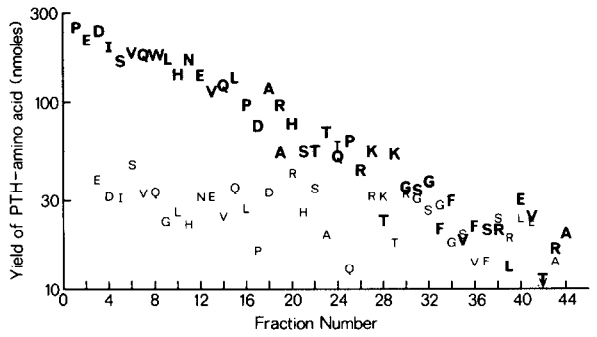

Fig. 1. Sequencer yields of ϵ2-fragment.

additives and the identification of PTH-amino acids were as described previously [8].

3. Results and discussion

The amino acid composition of fragment ϵ2 did not contain homoserine, indicating that ϵ2 is the C-terminal fragment of the ϵ-chain. The results of the ϵ2 degradation are summarised in fig. 1. The repetitive yield was about 94%. After the first few residues, the overlap sequence, displaced from the main sequence by one residue, can be followed.

The ϵ4 sequence was followed for 12 residues and gave the results shown in table 1.

Analysis of the tryptic and peptic peptides by the dansyl-Edman procedure permitted the elucidation of a continuous sequence accounting for the C-terminal half of the fragment (fig. 2). This, combined with the sequencer results gave a continuous sequence for the whole of the ϵ2 fragment in close agreement with the amino acid composition of the intact fragment. The C-terminal peptide was obtained in two forms in about equal yields: ϵ2TN2 which ends with glycine and ϵ2TB4b which ends with glycine–lysine. The amino acid analysis of intact ϵ2 also indicates the presence of a fractional amount of lysine. The reason for this heterogeneity at the C-terminus is obscure, but a similar heterogeneity has been observed in a haemoglobin variant [9]. It seems likely that this arises from a carboxypeptidase B-like activity in serum [10].

The tryptic peptides of the N-terminal half of ϵ2 were obtained in lower yields. This is probably due to the formation, during tryptic digestion, of an insoluble precipitate which is resistant to further cleavage. The

Table 1
Sequencer yields of ϵ4-fragment.

Fraction	Major		Minor**	
	Residue	(nanomoles)	Residue	(nanomoles)
1	Arg	400	–	–
2	Ser	> 400*	–	–
3	Thr	> 170*	–	–
4	Thr	> 170*	–	–
5	Lys	> 200*	–	–
6	Thr	100	Lys	32
7	Ser	370	–	–
8	Gly	> 310*	–	–
9	Pro	210	–	–
10	Arg	92	Pro	90
11	Ala	200	Arg	44
12	Ala	< 50		

* Peak off-scale on amino-acid analyser.
** No values given if the yield was lower than 20% of main residue.

tryptic peptide located between ϵ2TBX and ϵ2TN1 was never detected (see fig. 2). This peptide contains bonds very susceptible to chymotryptic activity which, together with the insolubility described above, may account for this failure.

One serine residue in the tryptic peptide ϵ2TB5 has been marked with an asterisk; during the dansyl-Edman procedure this residue gave only a minor amount of DNS-serine plus large amounts of a fluorescent component which could not be identified. The amino acid composition of the peptide was unequivocal, which together with the clear cut results obtained during the dansyl-Edman procedure permits an assignment of this residue by difference. The possibility that this particular serine residue is modified in some way is not excluded.

The sequence of fragment ϵ4 was more straightforward (fig. 3). All the tryptic peptides could be prepared in good yields and the automatic sequencer data were used for overlapping the first three tryptic peptides. In this case the automatic sequencer gave reasonable yields for only 12 cycles (fig. 1b) due to the solubility of the remaining fragment in the organic solvents.

Since ϵ2 and ϵ4 are formed from reductive cleavage of a single disulphide-bonded fragment, their unique half-cystines must form an intrachain loop. This indi-

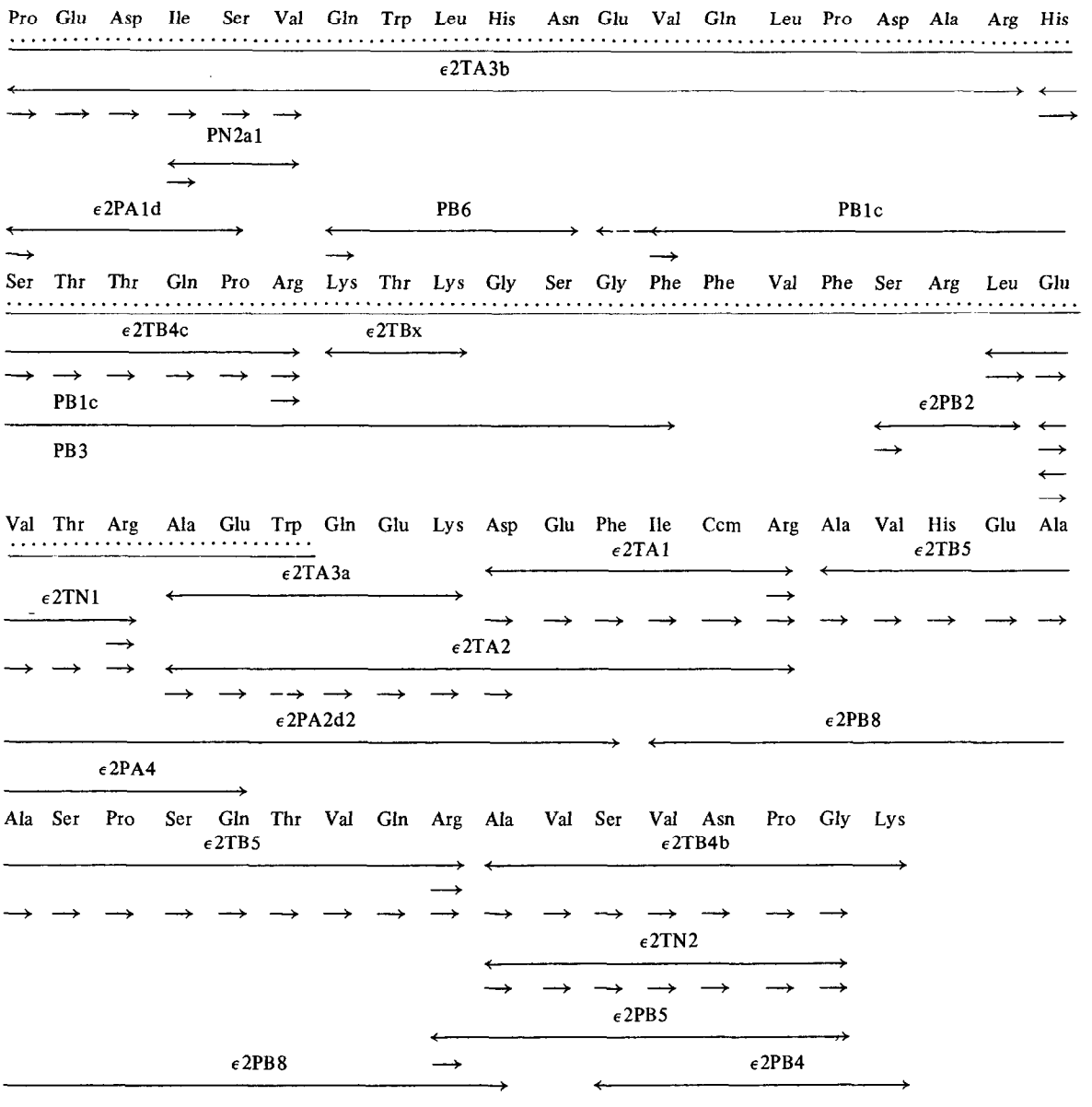

Fig. 2. The amino acid sequence of fragment ε2. The continuous line indicates the section of the peptide for which the automatic sequencer results are shown in fig. 1. Tryptic peptides (ε2T) and peptic peptides (ε2P) were subjected to the dansyl-Edman procedure as indicated by arrows. Double arrows indicate that the residue was obtained as a free amino acid at the end of the degradation.

cates that they belong to the same domain. When the sequences of the ε2 and ε4 fragments are compared with the sequence of the C-terminal part of human γ- and μ-chains, there are striking homologies (fig. 4), which suggests that ε4 and ε2 are contiguous and together account for the C-terminal domain of the ε-chain. The data further suggest that ε-chain arose early in the evolution of the immunoglobulin chains

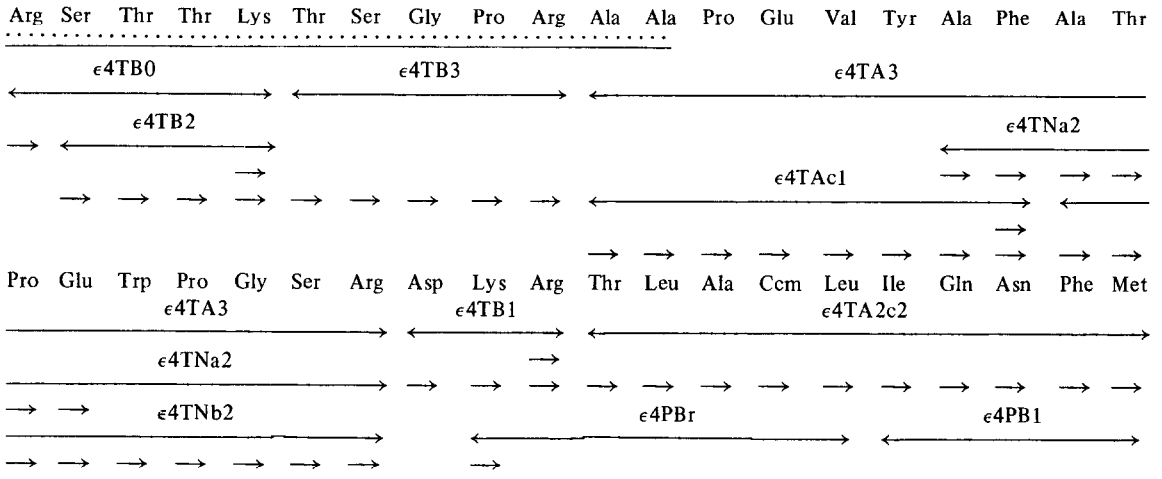

Fig. 3. Sequence of ϵ4 fragment. Details as for fig. 2. The two unique half-cystine of the unreduced ϵ4 and ϵ2 fragment are linked as an intrachain S–S bond.

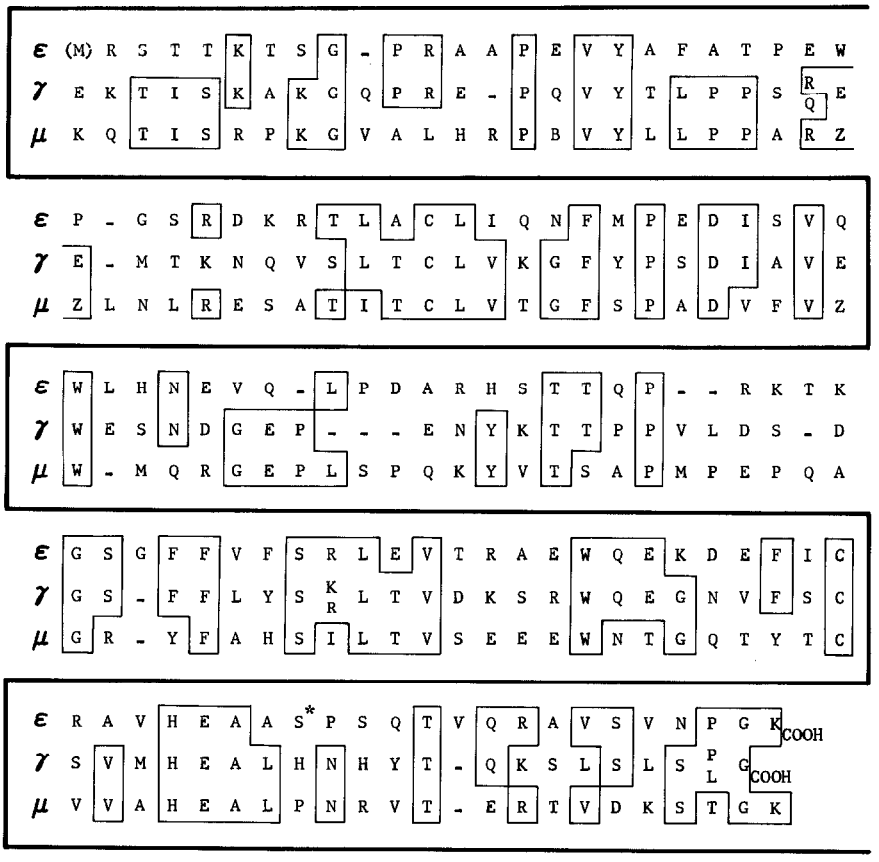

Fig. 4. Comparison of the sequence of fragments ϵ4 and ϵ2 with the $C_\gamma 3$-domain and the analogous region from μ-chain. The γ-chain line includes the alternative variants of γ1 [11] and γ4 [12] human sequences. The human μ sequence [13] includes some unpublished data kindly provided by Dr. F. Putnam.

Table 2

Differences between the C-terminal domain of ϵ-, γ- and μ-chains as compared in fig. 4. The differences are calculated as minimum number of base changes required to convert one sequence into the other. Gaps are computed as two base changes or one if it involves the carboxy-terminal residue.

	ϵ	γ
ϵ	–	
γ	112	–
μ	133	100

(table 2). The differences between each pair of the sequences of γ, μ and ϵ are reasonably close but the largest difference occurs between ϵ and μ suggesting that these two genes diverged first.

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