CHEMICAL TYPING OF HUMAN IMMUNOGLOBULINS E AND D

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1. Introduction

Immunoglobulins E and D (IgE and IgD)* are minor components of normal human sera and myeloma proteins of these classes are rarely found. The heavy (H) chain of IgE (ϵ chain) and of IgD (δ chain) have antigenic and physiochemical characteristics that distinguish them from other heavy chains [1–4]. Furthermore, it was shown that human atopic allergy is mediated by the reaginic antibodies of IgE specificity [5]. Using a previously developed procedure [6, 7], we report here the chemical typing of IgE and IgD and the sequences around S–S bonds mildly reduced and radioactively alkylated molecules. Well defined characteristics distinct from IgG, M, A and their subclasses were found.

2. Experimental

IgE, λ (Ps) was isolated from human myeloma sera as described [8]. IgD, λ (Men) myeloma protein was purified by starch block electrophoresis in 0.05 M barbital buffer (pH 8.6).

Fifty mg of each protein were dissolved at a protein concentration of 20 mg/ml in 0.27 M Tris-HCl buffer (pH 8.2) and reduced with 0.005 M dithiothreitol at room temp. under N_2. After 1 hr, reduction was terminated by the addition of iodo-[^{14}C]acetic acid (0.01 M, specific activity 0.7 mCi/mmole). The reaction was allowed to proceed for 1 hr at room temp., following which the proteins were dialyzed overnight against 5% formic acid.

Digestion with pepsin (Worthington, twice crystallized) enzyme/substrate ratio 1:50 (w/w), was carried out for 14 hr at 37°C, and after freeze drying the material was dissolved in 0.2 M ammonium bicarbonate, pH 8.3, and digested with L-(1-tosylamido-2-phenyl) ethyl chloromethyl ketone trypsin (Worthington) enzyme/substrate ratio 1:50 (w/w), for 14 hr at 37°C. The digest was dried, dissolved in water, and applied on 3 MM Whatman paper. A mixture of aspartic acid, glutamic acid, ϵ-DNP-lysine and glycylalanine was applied at the sides of the paper as a marker. The paper was then subjected to high voltage electrophoresis at pH 3.5 (pyridine–acetic acid–water, 1:10:190, v/v) for 1 hr, 60 V/cm. Peptides containing S-carboxymethylcysteine were detected by autoradiography, using Kodak Royal Blue Medical X-ray film.

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* The terminology we have used is that recommended by the World Health Organization (W.H.O. Bull., 33, 721 (1965); 35, 953 (1966); 38, 151 (1968); 41, 975 (1969).

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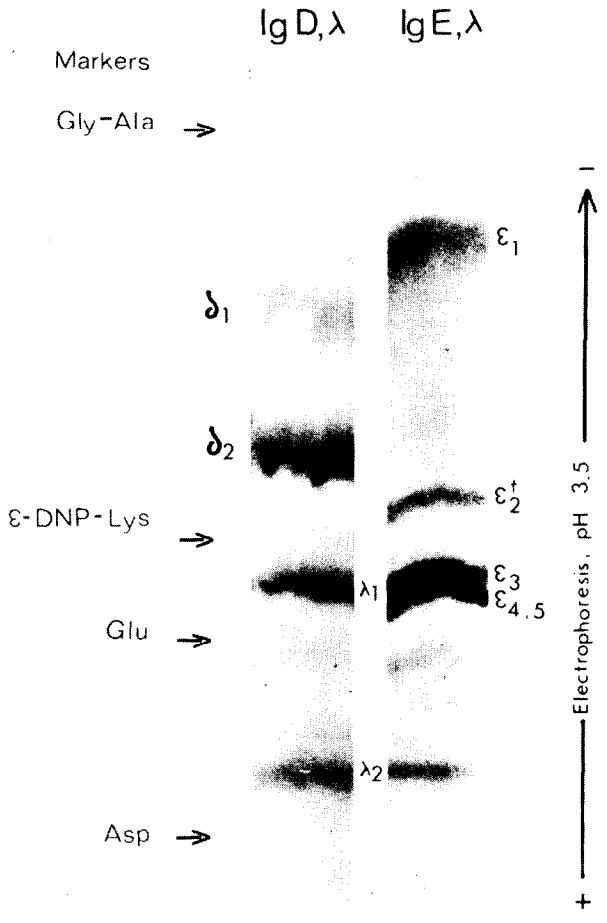

Fig. 1. Autoradiograph of the radioactive peptides from a peptic-tryptic digest of partially reduced and iodo-[^{14}C]acetic acid-labeled myeloma protein Men (IgD, λ) compared by electrophoresis at pH 3.5 with peptides similarly obtained from myeloma protein Ps (IgE, λ). λ1 and λ2 are two peptides derived from L chain (λ type). Peptides δ1 and δ2 derived from the H chain (δ) of IgD and peptides ε1, ε2, ε3, ε4 and 5 derived from the H chain (ε) of Ige. For sequence, see table 1. + A methionine containing peptide was also obtained from this region (table 1).

Table 1

Sequence of the main peptic-tryptic S-carboxymethylcysteine peptides obtained after partial reduction and alkylation of IgD, λ (Men) and IgE, λ (Ps) myeloma proteins.

Protein	Peptide	Mob. at pH 6.5*	Sequence
IgD (Men)	δ1	N	Pro–Ile–Ile–Ser–Gly–Cys–Arg
	δ2	0.37	Thr–Pro–Glx–Cys–Pro–Ser–His–Thr–Glx–Pro(Leu, Gly, Val)
	λ1	0.8	Pro–Thr–Glu–Cys–Ser
	λ2	0.72	Cys–Ser
IgE (Ps)	ε1	N	Thr–Cys–Arg
	ε2	0.62	Cys–Ala–Asx–Ser–Asx–Pro–Arg
	ε3	0.57	Gly–Cys–Leu
	ε4	0.47	Cys–Cys–Lys
	ε5	0.82	Ser–Val–Cys
	+	N	Leu(Gln, Met)
	λ1	0.8	Pro–Thr–Glu–Cys–Ser
	λ2	0.72	Cys–Ser

* Electrophoretic mobilities of peptides at pH 6.5 with respect to aspartic acid taken as 1.0.

+ S-carboxymethylhomocysteine containing peptide. For position on autoradiograph, see fig. 1.

3. Results and discussion

Fig. 1 shows the autoradiograph obtained after electrophoretic separation of a peptic-tryptic digest of partially reduced and carboxymethylated myeloma proteins IgD and IgE at pH 3.5. Four radioactive bands containing S-carboxymethylcysteine peptides are present in protein Men. Two of these, δ1 and δ2, belong to the H chain and the other two, λ1 and λ2, represent different degradation products of the carboxy end of the λ chain. The amino acid sequences of these CMCys peptides are shown in table 1. They are identical to similar peptides from another human IgD myeloma protein previously reported [9]. Peptic-tryptic diagonal electrophoresis at pH 6.5 [10] showed that peptides λ1 and λ2 were bridged to δ1, and δ2 was bridged to itself. Therefore, δ1 and δ2 peptides contain the half-cysteine residues responsible for H–L and H–H binding, respectively.

Five main radioactive bands are present in protein Ps (fig. 1): ε1; ε2; ε3; ε4,5, λ1; and λ2. Peptides ε4 and ε5 cannot be distinguished on one dimension because they run in the same position. Moreover, the carboxyl end of λ chain (band λ1) also runs in the same position. The amino acid sequence of all these peptides is shown in table 1. Peptides λ1 and λ2 are related and they belong to the carboxyl end of the λ chain. Five peptides are present on the ε chain and

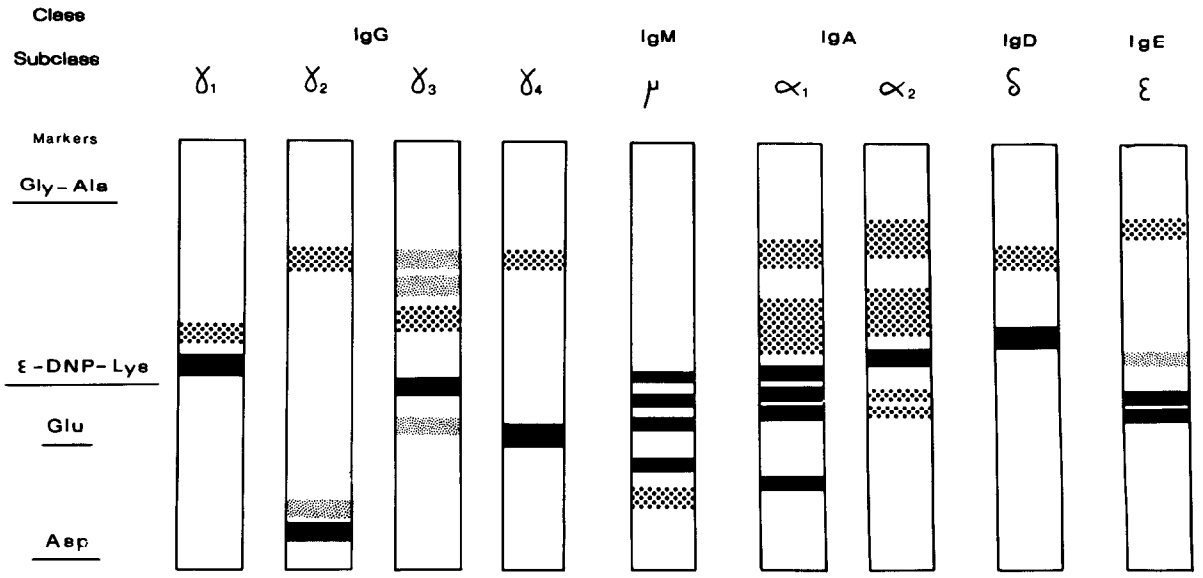

Fig. 2. Composite diagram of autoradiograph showing the position of radioactive bands containing the interheavy chain bridges of human IgG, M, A, D and E and their subclasses. γ, μ, α, δ and ϵ: heavy chain of IgG, M, A, D and E. $\gamma 1$, $\gamma 2$, $\gamma 3$ and $\gamma 4$: subclasses of IgG. $\alpha 1$ and $\alpha 2$ subclasses of IgA. The intensity of the bands is shown as seen on autoradiographs.

one of them contains two cysteine residues (peptide $\epsilon 4$). Although the function of these peptides is not known, presumably most of them are involved in interchain disulfide bridges.

Fig. 2 is a composite diagram of a radioautograph showing main radioactive bands containing the interchain disulfide bonds of all known classes and subclasses of immunoglobulin heavy chains. The distribution pattern depends upon the number of cysteines involved in interchain linkage and the differences in mobility depend upon the amino acid sequence around those bonds. Thus it is possible, at the present time, to recognize by simple inspection nine different classes and/or subclasses of immunoglobulin heavy chains.

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