SHORT COMMUNICATIONS

Fragmentation of human IgG globulin with papain, trypsin and pepsin

In a previous[1] paper we reported a simple procedure for the preparation of F_{ab} fragments from pure IgG immunoglobulin, consisting of a well-defined reduction followed by a short enzymatic digestion with papain.

Immunologically identical fragments can also be obtained after splitting with the proteases trypsin[2] and pepsin[3]. However these fragments need not necessarily also be identical chemically, since the enzymes used for the splitting of the globulins have different specificities. To investigate this matter, we have digested IgG with the three proteases, using the same procedure each time, and have compared the immunological properties and the amino acid composition of the fragments thus prepared.

A 2% solution of immunoelectrophoretically pure human IgG in 1 *M* Tris buffer pH 8.0 was reduced at room temperature by adding 2-mercaptoethanol to a final concentration of 0.02 *M*. After 30 min the solution was cooled to 0° and the free SH groups were blocked by adding an excess of N-ethyleneimine (0.4 *M*). The solution was subjected to gel filtration on Sephadex G-150 and eluted with a 0.1 *M* Tris–0.5 *M* NaCl–0.002 *M* EDTA buffer (pH 8.2) to isolate the "7 S" fraction (polymer and reagent fractions were rejected).

The solution of reduced IgG thus obtained was:

(a) for treatment with papain dialysed against 0.05 *M* acetate buffer (pH 5.5) for 16 h and digested for 4 h at 37° by cysteine-activated papain (Worthington, 2 × recrystallised) to which EDTA was added in the ratio protein–papain–cysteine–EDTA 100:1:0.6:3.7.

(b) For treatment with pepsin dialysed against 0.05 *M* acetate buffer (pH 4.0) for 16 h and digested for 4 h at 37° by pepsin (Worthington, 2 × recrystallised). Ratio protein–pepsin 100:1.

(c) For treatment with trypsin dialysed against 0.1 *M* Tris (pH 8.0) for 16 h and digested for 4 h at 37° by trypsin (Worthington 2 × recrystallised). Ratio protein–trypsin 50:1.

The digestion was stopped by freezing and thawing the solution, and in cases (a) and (b) followed by dialysis against large volumes of ice-cold water for 8 h; in case (c) followed by lowering the pH to 6.0 and dialysis against large volumes of ice-cold water for 8 h.

By gel filtration on Sephadex G-150 the 3.5 S fragments could be obtained from the digest, free of 7 S globulins, 5 S fragments, enzymes and lower molecular weight fractions (see Fig. 1). That our isolated fraction indeed consisted of molecules with a sedimentation coefficient of 3.5 S ± 0.1 was checked in the ultracentrifuge.

By chromatography of the 3.5 S fraction on DEAE Sephadex A-50, eluting buffer 0.01 *M* phosphate pH 8.0 (ref. 5), it was possible to isolate the F_{ab} fragment. Rechromatography of this fragment under the same conditions yielded immunoelectrophoretically pure F_{ab} fragments, as is seen in Fig. 2.

The three types of fragments thus obtained were compared with each other and with IgG globulin in the Ouchterlony double diffusion test and found to be identical (Fig. 3).

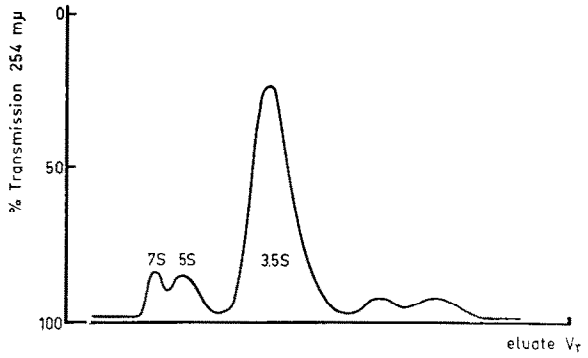

Fig. 1. Gel filtration of the enzyme digest on Sephadex G 150.

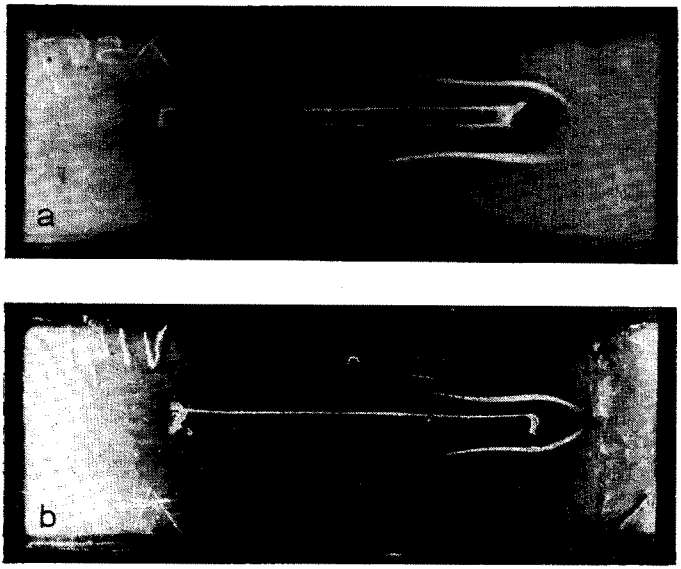

Fig. 2. Immunoelectrophoresis of IgG and F_{ab} fragments. The antiserum used is an anti-IgG serum. a. Lower: F_{ab} obtained with papain; Upper: IgG globulin. b. Lower: F_{ab} obtained with trypsin; Upper: F_{ab} obtained with pepsin.

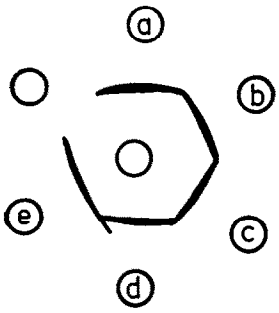

Fig. 3. Ouchterlony test of F_{ab} fragments. Center well: anti-IgG serum. a. F_{ab} papain; b. F_{ab} trypsin; c. F_{ab} pepsin; d. F_{ab} papain; e. IgG globulin.

Amino acid analyses were performed in duplicate as described earlier[4]. From our analyses, as presented in Table I, we see that small differences in amino acid composition indeed exist, notably in the content of serine, proline, tyrosine, arginine.

In conclusion: although the F_{ab} fragments are identical immunologically and have the same sedimentation coefficient, they show small differences in composition, which however do not affect their immunological identity.

TABLE I

AMINO ACID COMPOSITION OF IgG GLOBULINS AND F_{ab} FRAGMENTS, EXPRESSED AS g AMINO ACID PER 100 g PROTEIN

Amino acid	*IgG*	*F_{ab} pap*	*F_{ab} tryp*	*F_{ab} pep*
asp	8.05	8.15	7.93	8.31
thr	7.18	8.38	8.27	8.37
ser	9.60	11.06	9.91	11.73
glu	11.01	10.29	12.04	11.06
pro	6.87	5.68	6.54	6.45
gly	3.40	4.53	4.34	4.28
ala	3.38	4.31	4.31	4.68
val	7.89	8.08	7.75	8.09
met	0.87	0.78	0.77	0.95
ileu	2.11	2.73	2.36	2.61
leu	7.45	7.68	7.47	7.62
tyr	5.76	6.44	7.36	6.28
phe	4.37	4.42	4.12	4.39
lys	6.90	6.39	6.67	6.27
his	2.37	2.08	1.99	2.05
arg	4.03	4.56	4.14	5.34

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