Subfragments from the Fc Fragment of Human Immunoglobulin G

ISOLATION AND PHYSICOCHEMICAL CHARACTERIZATION

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A fragment termed fragment Fc' and a related fragment termed fragment pFc' produced by the actions of papain and pepsin respectively on human immunoglobulin G have been isolated and characterized. Amino acid analyses and experiments utilizing cyanogen bromide to cleave the methionyl bonds of the Fc' and pFc' fragments make it possible to locate both fragments within the known chain structure of the immunoglobulin G molecule. The pFc' fragment is probably a non-covalently linked dimer situated at the C-terminal end of the molecule, containing about 232 amino acid residues and having a molecular weight of 26 000. The Fc' fragment is a similar dimer of about 182 residues extending from near residue 14 to near residue 105 (numbered from the C-terminal end) of the γ -chain and has a molecular weight of 21 000.

The work of Porter (1959) showed that papain split the IgG† molecule into two distinct types of fragment, now called fragments Fc and Fab in the nomenclature recommended by the World Health Organisation (1964). Nisonoff, Wissler, Lipman & Woernley (1960) demonstrated that pepsin behaved somewhat differently and gave a single major fragment, called fragment F(ab')₂.

In the past there has been little interest in the minor fragments that are also liberated during enzymic attack. Both papain and pepsin give rise to non-diffusible fragments that are related to the Fc fragment. With papain the presence of one such component was revealed in the immunoelectrophoretic studies of Edelman, Heremans, Heremans & Kunkel (1960), and this was called fragment F' by Fahey & Askonas (1962); the same fragment was called fragment F'c by Poulik (1966). Turner & Rowe (1966) reported the presence of this fragment in normal human urine and referred to it as fragment Fc', since this seemed more consistent with current immunoglobulin nomenclature.

Following work by Goodman (1963), Utsumi & Karush (1965) demonstrated the release of fragments related to the Fc fragment of rabbit IgG during pepsin digestion; one such fragment was termed fragment Pep III'. While the present work was in progress Prahl (1967) reported the location of fragment Pep III' within the structure of rabbit Fc fragment, and a counterpart from the human Fc

fragment (termed component II) was reported by Heimer, Schnoll & Primack (1967).

The present paper is concerned with a comparison of the physicochemical characteristics of the human Fc' fragment and the pepsin fragment of human IgG which we have shown to have a related structure and have accordingly designated 'pFc' fragment'. Finally we have studied the disposition of both fragments in the molecular chain structure of IgG as proposed by Porter (1962).

MATERIALS AND METHODS

Human IgG. A Cohn fraction II preparation of pooled human IgG was kindly supplied by Kabi AB, Stockholm, Sweden.

Preparation of Fc' fragment. Papain digestion of whole IgG was performed as described by Porter (1959), with mercuripapain from Sigma Chemical Co., St Louis, Mo., U.S.A. The reaction was terminated by the addition of iodoacetamide to give a final concentration of 10 mM, and the digest was fractionated by preparative column electrophoresis (Porath, 1956). After dialysis against 30 mM-barbitone buffer, pH 8.6, with $\frac{3}{8}$ Visking tubing, the sample was applied to an electrophoresis column (type 5800; LKB-Produkter, Stockholm, Sweden) packed with cellulose (no. 400; Munktell, Grycksbo, Sweden) and equilibrated with 0.1 M-barbitone buffer, pH 8.6, containing 0.02% (w/v) NaN₃. The column was maintained at 4–6° and a current of 400 mA (600 V) was applied for 71 hr. After electrophoresis the column was eluted with buffer (see Fig. 1) and fractions shown to contain Fc' fragment by immunoelectrophoretic analysis were pooled, dialysed against water with $\frac{3}{8}$ Visking tubing and freeze-dried. The recovered material represented approx. 4–5% by wt. of the starting IgG. It was redissolved

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† Abbreviation: IgG, immunoglobulin G.

Fig. 1. Isolation of Fc' fragment by column electrophoresis. A 6% (w/v) solution of papain-digested human IgG (100 ml.) was subjected to electrophoresis on a cellulose column equilibrated with 0.1M-barbitone buffer, pH 8.6. The distribution of Fc' , Fc and Fab fragments and of light chains (L), which was determined by immunoelectrophoresis of the effluent fractions, was as shown. The fractions, indicated by the horizontal bar, that contained mainly Fc' fragment were pooled.

Fig. 2. Recycling on a 3.2 cm. $\times$ 98 cm. column of Sephadex G-50 of 5 ml. of the Fc' solution isolated by column electrophoresis (see Fig. 1). The sample was recycled three times as indicated by the inset, which shows the transmission at 254 $\text{m}\mu$ (T). At positions 1 the column system was closed to allow effluent to pass directly into the column, at positions 2 the system was opened for elution. Fractions (3.3 ml.) were collected. The fractions indicated by the horizontal bar were pooled to give Fc' fragment.

in 5 ml. of 0.1 M-tris-HCl-0.2 M-NaCl-2 mM-EDTA (disodium salt), pH 7.7, containing 0.02% NaN_3 . The sample was then applied to a 3.2 cm. $\times$ 98 cm. column of Sephadex G-50 (particle size 20–80 μ) (Pharmacia, Uppsala, Sweden) and subjected to recycling in the same tris buffer (Porath & Bennich, 1962). The column was operated at a flow rate of approx. 2 cm./hr. and a temperature of 20–22°. Fig. 2 shows the result of cycling the material four times. Two peaks were resolved and 'bleeds' were made on cycles 2 and 3 to remove the first peak, which contained intact Fc fragment. On the fourth cycle the second peak (containing Fc' fragment) was eluted. This material was pooled, dialysed against water with $\frac{2}{3}$ Visking tubing and concentrated either by freeze-drying or by ultrafiltration in collodion bags (Membranfiltergesellschaft, Göttingen, Germany).

Preparation of pFc' fragment. Pepsin digestion of human IgG was performed in a manner similar to that described by Utsumi & Karush (1965) for rabbit IgG. Digestion of a

Fig. 3. Recycling on a 3.2 cm. $\times$ 98 cm. column of Sephadex G-50 of 5 ml. of pFc' solution isolated by gel filtration on Sephadex G-150. The sample was recycled three times as indicated by the inset, which shows the transmission at 254 $\text{m}\mu$ (T). At positions 1 the column system was closed to allow effluent to pass directly into the column; at positions 2 the system was opened for elution. Fractions (3.0 ml.) were collected. The fractions indicated by the horizontal bar were pooled to give pFc' fragment.

3% (w/v) solution of IgG in 0.1 M-sodium acetate buffer, pH 4.5, was carried out at 37° for 24 hr. with twice-crystallized pepsin (Worthington Biochemical Corp., Freehold, N.J., U.S.A.). The enzyme was dissolved in buffer immediately before the digestion and used at an enzyme:substrate ratio 1:100 by wt. The reaction was terminated by adjusting the pH to 8.0–8.2 with solid tris. The digestion mixture was fractionated on a 7 cm. $\times$ 112 cm. column of Sephadex G-150 (particle size 40–120 μ), equilibrated with 0.1 M-tris-HCl-0.2 M-NaCl-2 mM-EDTA (disodium salt), pH 7.7, containing 0.02% (w/v) NaN_3 , and operated at a flow rate of approx. 1.5 cm./hr. The second peak to be eluted (cf. Heimer *et al.* 1967, Fig. 2) contained the pFc' fragment, which was identified by immunoelectrophoresis, and this material (representing approx. 9–10% by wt. of the starting IgG) was pooled, dialysed against water and freeze-dried. The freeze-dried material was redissolved in the same tris buffer to give a volume of 5 ml., and subjected to recycling on Sephadex G-50 to remove contaminating Fab' fragments. Fig. 3 shows the result of cycling the material four times. Two peaks were resolved and 'bleeds' were made on cycles 2 and 3 to remove the first peak. On the fourth cycle the major peak, containing the pFc' fragment, was eluted. This material was pooled, dialysed against water and concentrated by either freeze-drying or ultrafiltration.

Papain digestion of pFc' fragment. Preparations of pFc' fragment were adjusted to 1% (w/v) in tris buffer, pH 7.7 (see above). Cysteine hydrochloride was added to a final concentration of 10 mM and the substrate solution was mixed with mercuripapain to give an enzyme:substrate ratio 1:200 by wt. The mixtures were incubated at 37° for 12 hr. and the reaction was terminated by the addition of iodoacetamide solution (0.1 mg./ml. of reaction mixture) before application to a column of Sephadex G-50.

Ultracentrifugal analysis. Sedimentation-velocity studies were made in a Spinco model E ultracentrifuge at 59780 rev./min. in a 12 mm. 2° double-sector cell at 20°. Sedimentation coefficients were calculated by extrapolation to zero protein concentration and corrected for density and viscosity of the solvent (Svedberg & Pedersen, 1940).

Sedimentation-equilibrium studies were made in a 12 mm. 2° double-sector cell at 12590 rev./min. at 20°. The concentration of the samples was previously determined in a synthetic-boundary cell at 10000 rev./min. Molecular weights were calculated from measurements at different protein concentrations by the Archibald method as described by Ehrenberg (1957), assuming a partial specific volume 0.749.

Amino acid analysis. Samples (1–2 mg.) of Fc' and pFc' fragments were hydrolysed for 24 and 72 hr. as described by Von Hofsten, Van Kley & Eaker (1965) and analysed according to the procedure of Spackman, Stein & Moore (1958) in a Spinco model 120B amino acid analyser equipped with an Infotronics model CRS 124B electronic integrator. Total cystine plus cysteine was determined as cysteic acid after oxidation with performic acid (Moore, 1963). Tryptophan was determined colorimetrically (procedure N) as described by Spies & Chambers (1949). The optimum conditions for colour production (a reaction time of not less than 30 hr. at 20° in the dark) were evaluated with human IgG.

Cyanogen bromide treatment of Fc' and pFc' fragments. Samples of Fc' fragment (59 mg.) and pFc' fragment (80 mg.) were incubated with a twofold excess of CNBr (Fluka A.-G., Buchs SG, Switzerland) in 70% (v/v) formic acid for 24 hr. at 25°, as described by Givol & Porter (1965). The products were freeze-dried, redissolved in N-acetic acid and subjected to gel filtration on a 3.2 cm. × 96.2 cm. column of Sephadex G-50 equilibrated in the same solvent. Material eluted in the same position as the octadecapeptide released from whole human IgG (Press, Piggot & Porter, 1966b) was concentrated by freeze-drying.

Carbohydrate analysis. Hexose was determined by an orcinol-H₂SO₄ method (Svennerholm, 1956) with D-galactose as standard. Sialic acid was determined by the thiobarbituric method as described by Warren (1959) with N-acetylneuraminic acid as standard; before analysis 1 mg. samples in 0.1 ml. of water were hydrolysed with 0.1 ml. of 0.2 N-H₂SO₄ at 100° for 70 min.

Reduction of Fc' and pFc' fragments. Solutions (1%, w/v) of Fc' and pFc' fragments in 0.1 M-tris-HCl buffer, pH 7.7, containing 2 mM-EDTA (disodium salt) were treated with β-mercaptoethanol (final concentration 0.2 M) for 1 hr. at 20–22°. The reduced samples were compared with untreated fragments by starch-gel-electrophoretic analysis in urea buffers at pH 3.0 and pH 7.8.

Starch-gel electrophoresis. Horizontal starch-gel analyses (Wake & Baldwin, 1961) were made in urea buffer, pH 3.0, containing 8 M-urea in 50 mM-formate–10 mM-NaOH

(Edelman & Poulik, 1961), and in urea buffer, pH 7.0–8.0, containing 8 M-urea in 35 mM-glycine adjusted to pH 8.9 with NaOH (Cohen & Porter, 1964). Approx. 40 μl. samples containing 5–10 mg./ml. were applied on Whatman no. 3MM paper (6 mm. × 14 mm.) inserted in the gel. A voltage of 3–4 v/cm. was applied for 18–24 hr. at 20–22°. Gels were stained with 0.01% (w/v) Nigrosin in methanol-acetic acid-water (5:1:4, by vol.) and destained in the same solvent.

Immunoelectrophoresis. This was performed by the method of Grabar & Williams (1953) with 1.5% (w/v) Special Difco Noble agar in barbitone buffer, pH 8.6 and I 0.05.

Antisera. Antisera to whole human IgG and to light and heavy polypeptide chains of IgG were raised in rabbits by intramuscular injection of antigen (0.5–1 mg.) in complete Freund's adjuvant. Second injections were given after 14 days with subsequent injections at weekly intervals for 3 weeks. The animals were bled 5 days after the last injection.

RESULTS

The isolated Fc' and pFc' fragments produced precipitin arcs of characteristic mobility when examined by immunoelectrophoretic analysis (Fig. 4a). There was no evidence of contamination with other fragments in either case. However, starch-gel electrophoresis (Fig. 4b, samples 1–4) showed that both fragments were still electrophoretically heterogeneous, particularly in the alkaline gel (samples 3 and 4).

Figs. 4(a) and 4(b) also show the effect of digesting pFc' fragment with papain. Immunoelectrophoresis of a papain digest of pFc' fragment revealed a precipitin band having a mobility very similar to that of Fc' fragment. Similarly, starch-gel electrophoresis at both pH 3.0 and pH 7.0 of such material (Fig. 4b, samples 6 and 8) revealed bands having mobilities similar to those given, under the same conditions, by Fc' fragment isolated from IgG.

Further, gel filtration on Sephadex G-50 of papain-digested pFc' fragment gave two peaks. The first peak was eluted in a volume identical with that required for the Fc' fragment and the second peak was eluted close to the total column volume. The material in the first peak was shown by immunoelectrophoresis, starch-gel electrophoresis and amino acid analysis to be indistinguishable from the Fc' fragment. It was concluded that the Fc' fragment must represent a major part of the pFc' fragment.

Ultracentrifugation of Fc' and pFc' fragments. Fig. 5 shows the effect of concentration on the sedimentation coefficient of two preparations of Fc' fragment and one preparation of pFc' fragment. Extrapolation to zero concentration gave $S_{20,w}^0$ values of 2.35 s for the Fc' fragment and 2.38 s for the pFc' fragment.

Molecular-weight determinations by the

Fig. 4. (a) Immunelectrophoresis of isolated Fc' and pFc' fragments compared with whole papain and pepsin digests of IgG, with an antiserum to whole human IgG. The bottom well contained papain-digested fragment pFc'. (b) Starch-gel-electrophoretic analyses showing isolated fragment Fc' (1 and 3), isolated fragment pFc' (2 and 4), untreated fragment pFc' (5 and 7) and papain-digested fragment pFc' (6 and 8). Samples 1, 2, 5 and 6 were run in 8M-urea-formate buffer, pH 3.0, and samples 3, 4, 7 and 8 in 8M-urea-glycine buffer, pH 7.0.

Archibald method gave values of 23400 for one preparation of Fc' fragment and 21500 for another preparation. A molecular weight of 26700 was obtained for the pFc' fragment.

Fig. 5. Effect of concentration on the sedimentation coefficient of two preparations of Fc' fragment (○ and △) and one preparation of pFc' fragment (□). Analyses were made in 0.15M-NaCl, pH 7.0.

Amino acid composition of Fc' and pFc' fragments. Table 1 shows the amino acid composition of Fc' fragment (average composition of three samples) and pFc' fragment. The three samples of Fc' fragment (two samples were prepared directly from IgG and the third by papain digestion of pFc' fragment) analysed showed excellent agreement for all amino acids except histidine; the sample prepared by papain digestion of pFc' fragment had significantly less histidine (2.4 residues/14 residues of leucine) than the samples prepared directly from IgG (3.0–3.4 residues/14 residues of leucine). The significance of this observation is discussed below.

Table 1 also shows the number of residues for each amino acid rounded off to the nearest even integer, on the assumption that the fragments were symmetrical dimers and that every amino acid must have been represented on both heavy chains. This assumption draws on data from the other investigations reported in this paper, i.e. reduction experiments, cyanogen bromide cleavage, carbohydrate analysis and molecular-weight determinations. The amino acid differences between the Fc' and pFc' fragments (Table 1) were in agreement with the conclusion that the pFc' fragment incorporates the Fc' fragment within its structure.

Carbohydrate analysis. The carbohydrate content of the whole IgG was: hexose, 1.2%; N-acetylneuraminic acid, 0.22%; N-acetylglucosamine, 1.3%. Hexose was found to be absent from both Fc' and pFc' fragments, less than 0.1 mole of N-acetylneuraminic acid was found/mole of the fragments, and no hexosamine was detected in either fragment as evaluated from the amino acid analyses of the 24 hr. hydrolysates.

Cyanogen bromide cleavage of Fc' and pFc' fragments. After cyanogen bromide treatment a peptide was isolated from pFc' fragment by gel filtration on Sephadex G-50 in N-acetic acid. Table 2 shows the amino acid composition of this peptide and, for comparison, that of the C-terminal octadecapeptide of human γ -chains determined by

Table 1. *Amino acid composition of Fc' and pFc' fragments*

Amino acid composition					Differences in even integers between Fc' and pFc' fragments
Fc' fragment		pFc' fragment			
*Residues/14 residues of leucine	Nearest even integer	Residues/18 residues of leucine	Nearest even integer		
Ala	4.3	4	6.4	6	2
Arg	5.7	6	5.9	6	0
Asp	18.6	18	22.1	22	4
Cysteic acid†	4.0	4	4.5	4	0
Glu	25.4	26	29.3	30	4
Gly	8.8	8	12.1	12	4
His	2.9	2 or 4	5.6	6	2-4
Ile	2.1	2	4.3	4	2
Leu	14.0	14	18.0	18	4
Lys	10.0	10	16.7	16	6
Met	3.3	4	4.2	4	0
Phe	7.7	8	8.3	8	0
Pro	16.0	16	19.1	20	4
Ser‡	20.1	20	29.2	30	10
Thr‡	12.0	12	15.3	16	4
Trp§		4		4	0
Tyr	7.8	8	10.4	10	2
Val	15.4	16	16.8	16	0
Total residues		182-184		232	

* Average composition of three samples; two samples were prepared directly from IgG and the third by papain digestion of pFc' fragment.

† Acid hydrolysates (18 hr.) of samples oxidized with performic acid (Moore, 1963).

‡ First-order extrapolation to zero-time hydrolysis.

§ The values were 4.7 moles/mole of Fc' fragment (mol. wt. 21 500) and 4.2 moles/mole of pFc' fragment (mol. wt. 26 700), as determined by the colorimetric method (procedure N) of Spies & Chambers (1949).

Press *et al.* (1966b). The cyanogen bromide peptide of pFc' fragment had an elution position from a column of Sephadex G-50 in *N*-acetic acid and an amino acid composition similar to those of the octadecapeptide. These observations taken together with the agreement between the observed and theoretical yield of the peptide (12 and 15.5% respectively, relative to a molecular weight of 26 700) strongly indicated that the *C*-terminal ends of the pFc' fragment and of γ -chain were identical.

After cyanogen bromide treatment of the Fc' fragment no material was recovered that could be identified as the octadecapeptide, and it was concluded that the *C*-terminal sequence of the Fc' fragment differed from that of the pFc' fragment.

Reduction of Fc' and pFc' fragments. Reduced and unreduced preparations of Fc' and pFc' fragments were examined by starch-gel electrophoresis at pH 3.0 and pH 8.0. No striking change in mobility or general pattern was observed. Thus there was no evidence of any interchain bond cleavage having taken place during the reduction of either fragment as one might expect to see represented by changes of mobility in the acid gel.

DISCUSSION

A detailed study of the Fc' fragment of human IgG was prompted by its discovery in human urine (Turner & Rowe, 1966). Chemical characterization of the urinary material would, however, be extremely difficult in view of the extensive range of other proteins and fragments present (Schultze & Heremans, 1966). Accordingly, in the present study serum IgG was used as the starting material and the Fc' fragment was obtained by electrophoresis of papain-digested IgG. Poulik (1966) used an electrophoretic approach to prepare Fc' fragment and claimed to have obtained pure material by simple block separation, but in our experience some further purification (i.e. recycling gel filtration) was necessary if all traces of the Fc fragment were to be removed.

The physicochemical data for the fragments were in good agreement with the observations of other authors. Thus the sedimentation coefficient of 2.4 s ($S_{20,w}^0$ 2.38 s) calculated for pFc' fragment was in agreement with values reported for this fragment from both rabbit (Utsumi & Karush, 1965) and

Table 2. Amino acid composition of a cyanogen bromide peptide from pFc' fragment

The results given for the C-terminal octadecapeptide of human γ -chain are those reported by Press *et al.* (1966b). N.D., Not determined.

	Amino acid composition		
	CNBr peptide from pFc' fragment		Octadecapeptide of human γ -chain (moles/mole)
	Residues/3 residues of leucine	Nearest integer	
Ala	1.1	1	1
Arg	0.2	—	—
Asp	1.1	1	1
Cys	—	—	—
Glu	2.5	2	2
Gly	1.1	1	1
His	2.8	3	3
Ile	0.1	—	—
Leu	3.0	3	3
Lys	1.3	1	1
Met	—	—	—
Phe	—	—	—
Pro	1.4	1	1
Ser	3.2	3	3
Thr	1.2	1	1
Trp	N.D.	—	—
Tyr	1.0	1	1
Val	0.2	—	—
Total residues		18	18

human IgG (Heimer *et al.* 1967). The molecular weight of 26700 for the pFc' fragment was also similar to values of 27500 and 25000 reported by these authors. In contrast, the sedimentation coefficient of the Fc' fragment ($S_{20,w}^0$ 2.35s) was a little higher than the value of 2.1s reported by Poulik (1966). No values for the molecular weight of the Fc' fragment have previously been reported.

Hexose, hexosamine and *N*-acetylneuraminic acid were shown to be absent from both the Fc' and the pFc' fragments. The absence of hexose from human Fc' fragment was reported by Poulik (1966), and in the rabbit Utsumi & Karush (1965) found less than 0.4 mole of hexose/27000g. of Pep III' fragment.

No evidence was found in the reduction experiments for the existence of an interchain disulphide bond in either the Fc' or the pFc' fragment. There were nevertheless several pointers that indicated that both fragments must be dimers, presumably non-covalently linked. Thus the molecular weight of the pFc' fragment was too large for it to be accommodated as a single chain within the Fc fragment, particularly since the carbohydrate and

Fig. 6. Schematic diagram of the Fc region of human IgG, showing the probable disposition of the Fc' and pFc' fragments. The location of the disulphide bridges and the carbohydrate side chains (CHO) are assigned by analogy with the structure of rabbit Fc fragment (Hill, Lebovitz, Fellows & Delaney, 1967). The amino acid residues are numbered from the C-terminal end of the γ -chains.

certain of the antigenic determinants of the Fc fragment were absent from the pFc' fragment. In addition, the yield of the C-terminal octadecapeptide from the pFc' fragment indicated that the fragment must have been a dimer. Poulik (1966) and Utsumi & Karush (1965) also concluded that human Fc' fragment and rabbit Pep III' fragment respectively were non-covalently linked dimers.

The results of cyanogen bromide cleavage taken together with the amino acid data made it possible to locate both the Fc' and pFc' fragments within the IgG molecule. This is illustrated schematically in Fig. 6. Only the Fc part of the four-chain structure described by Porter (1962) is shown. The pFc' fragment contained some 232 amino acids including the C-terminal octadecapeptide (Press *et al.* 1966b), and must therefore have been a dimer of about 116 amino acid residues located as shown. (Contrary to convention, amino acid residues are numbered from the C-terminal end of the γ -chain.) This is in close agreement with the recently published data of Prah (1967), who showed that rabbit fragment Pep III' occupied the C-terminal portion of the heavy chains of IgG with its *N*-terminal residue at position 113 from the C-terminal end. Prah (1967) also described a papain fragment derived from the rabbit Fc fragment and concluded that this was similar to the Pep III' fragment. It should be noted, however, that this was produced under acid conditions (pH 3.4–pH 3.7), in contrast with the present Fc' fragment, which was isolated at pH 6.8.

The position of the Fe' fragment cannot be as readily established, as it lacks the intact octadecapeptide. However, an indication of its probable position within the molecular structure is made possible by the close proximity of the histidyl residues within the octadecapeptide. The pFe' fragment contained six histidyl residues, all of which were located within the C-terminal octadecapeptide:

18 14 12
(Met)-His-Glu-Ala-Leu-His-Asn-His-Tyr-Thr-Gln-Lys-Ser-Leu-Ser-Leu-Ser-Pro-Gly

In contrast (see Table 1), the Fc' fragment may have had two or four histidyl residues. Thus the histidyl residue at position 12 is likely to have been absent whereas the histidine at position 18 must always have been present. However, although the histidine at position 14 was probably present in two preparations of Fc' fragment it was probably absent from Fc' fragment produced by papain digestion of pFc' fragment. This implied that this is a susceptible region from the point of view of papain action, with the actual point of cleavage depending on the conditions of digestion and the nature of the starting substrate. We therefore conclude that the Fc' fragment probably represented a non-covalently linked dimer extending from near residue 14 to about residue 105. Thus the C-terminus of the Fc' fragment should yield the peptide

18 14
His-Glu-Ala-Leu-His

after cyanogen bromide treatment, but we were unable to isolate and characterize such a peptide because of insufficient Fc' fragment.

If the approximate disposition of the Fe' fragment shown in Fig. 6 is correct, then about 11 amino acid residues (i.e. approx. residues 106-116) were lost when pFe' fragment was converted into Fe' fragment. The sequence in this region is not yet evaluated for human Fe fragment, but the sequence for rabbit Fe fragment is available (Hill, Delaney, Lebovitz & Fellows, 1966):

116 106
-Pro-Ile-Glu-Lys-Thr-Ile-Ser-Lys-Ala-Arg-Gly-

The residues 106–116 from rabbit Fc fragment and residues 1–13 from human Fc fragment account for all the amino acid differences between the pFc' and Fc' fragments except for alanine and isoleucine, both of which are 'deficient' in human pFc' fragment (Table 1). Thus the structural homology between species that Press, Givol, Piggot, Porter & Wilkinson (1966*a*) described for the octadecapeptide may also apply to other areas of the Fc fragment.

Finally, it should be noted that the range of genetic and biological properties associated with the

Fc fragment of IgG is large, and it will be desirable to investigate both the pFc' and Fc' fragments for such activities. In this way it may be possible to localize more precisely some of these functions of the Fc fragment of IgG.

Note added in proof. Since this manuscript was revised, Grey & Abel (1967) have produced evidence that both histidine and leucine are C-terminal amino

acids in human Fc' fragment. This agrees with our provisional placing of the fragment since, in the octadecapeptide, residue 14 is histidine and residue 15 is leucine.

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