A FRAGMENT CORRESPONDING TO THE C_H2 REGION OF IMMUNOGLOBULIN G (IgG) WITH COMPLEMENT FIXING ACTIVITY*

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1. Introduction

The Fc region of IgG, comprising approximately the C-terminal half of the two heavy chains mediates most of the effector functions of the molecule (e.g. interaction with the complement system, cytophilic activity, placental transfer, etc.). A considerable body of evidence [1, 2] suggests that the heavy and light chains of IgG are folded into a series of 'compact domains' comprising some 110 residues and each containing a single intrachain disulphide bond. Each heavy chain is folded into four such domains; the two C-terminal domains of each chain (designated C_H2 and C_H3) form the Fc region. Fragments corresponding to the C_H3 domain have been isolated and characterized [3, 4] and shown to be involved in the heterocytotropic activity of IgG [5]. Exposure of IgG to acid conditions has been shown to transiently render the region between C_H2 and C_H3 susceptible to proteolytic attack [6]. This approach has been used with Fc to prepare fragments related to C_H2 which are capable of interacting with complement.

2. Materials

Human IgG1 myeloma proteins were isolated and Fc fragments prepared from them by plasmin digestion following previously published methods [7, 8]. Trypsin (Type XI-DCC treated) and soybean trypsin inhibitor (Type 1-S) were obtained from Sigma Chemical Co. Antisera to Fc were prepared by absorbing rabbit antiserum to γ-chain with Fab fragment. Specific anti-C_H2 serum was obtained by absorbing anti-Fc with pFc' fragment ($\equiv C_H3$) prepared according to Turner and Bennich [3]. Dried guinea pig serum complement and sheep red cells were purchased from Connaught Medical Research Labs; rabbit hemolysin from Grand Island Biologicals and polystyrene latex particles (dia., 0.091 μm) from Dow Chemical Co.

3. Methods and results

Fc (20 mg/ml) in 0.15 M NaCl, 10 mM Tris-HCl, pH 7.8 was titrated to pH 2.5 with 2 N HCl at 25°. After 5 min at this pH, sufficient trypsin solution (10 mg/ml in 1.0 mM HCl) was added to give an enzyme to substrate ratio of 1 : 25 and the pH was rapidly readjusted to 7.8 with 2 M Tris. Serial samples were removed between 10 sec and 5 min and the trypsin inactivated with an equal weight of soybean inhibitor. Acid–urea starch gel electrophoresis showed that Fc had been cleaved. The optimum digestion time was near 45 sec and this time was used in subsequent experiments. Samples of Fc incubated at pH 7.8 with trypsin for up to 3 hr, without prior exposure, showed no cleavage. Similarly, Fc adjusted to pH 2.5 for 5 min and returned to pH 7.8, in the absence of trypsin, showed no fragmentation.

The digestion products of Fc were separated on a

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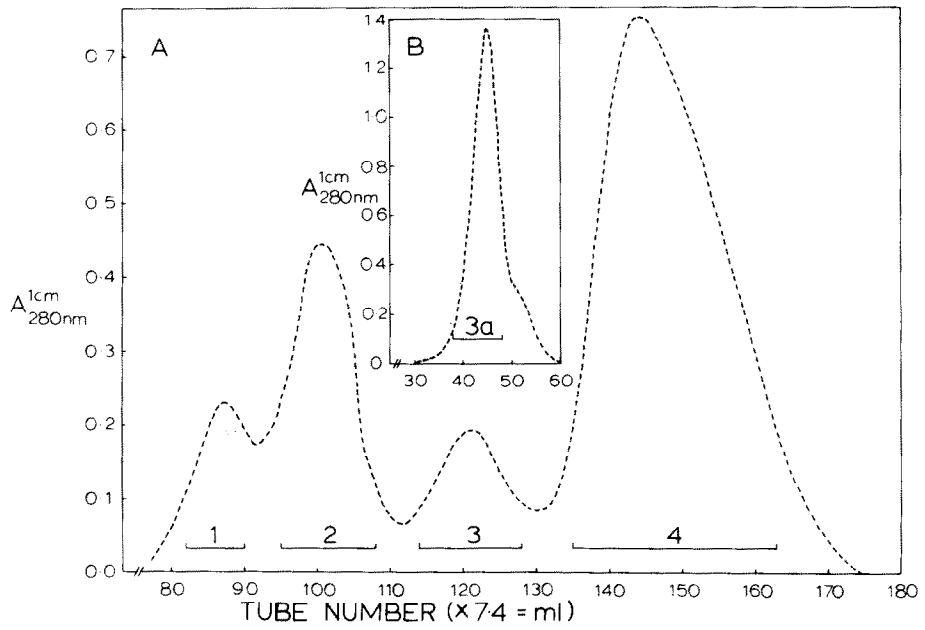

Fig. 1. A) Chromatography of a tryptic digest of acid-treated Fc on a 90.0 × 5.0 cm column of Sephadex G-100 in 1.0 M acetic acid, 25 mM NaCl. The effluent was pooled into four fractions as indicated by the horizontal lines. B) Rechromatography of fraction 3 under similar conditions. The main peak was pooled, as indicated, and the protein used for detailed analysis.

column of Sephadex G-100 (90 × 5 cm) in 1.0 M acetic acid, 25 mM NaCl as shown in fig. 1. Four protein peaks were resolved and the fractions pooled as indicated in fig. 1 before dialysis against 100 mM acetic acid, pH 3.2. In earlier experiments residual tryptic activity was apparent at this stage. To prevent further digestion soybean trypsin inhibitor coupled to Sepharose, according to the method of Cuatrecasas [9], was added prior to further dialysis against 0.15 M NaCl, 10 mM Tris, pH 7.8. The Sepharose was removed by centrifugation and the solutions concentrated by ultrafiltration.

Preliminary characterisation of fractions 1 to 4 was achieved on acid–urea starch gels (fig. 2a). Fraction 1 contained undigested Fc and two more rapidly migrating components which were also present in fraction 2. Other data (to be published) suggest that these represent intermediate digestion products. Fractions 3 and 4 each contained essentially single components. Prior to further analysis fractions 3 and 4 were separately rechromatographed on G-100 in 1.0 M acetic acid, 25 mM NaCl and the protein in the main peak dialysed against 0.15 M NaCl, 10 mM Tris, pH 7.8 (fraction 3a shown in fig. 1). Fractions 3 and 4 gave reaction of antigenic non-identity on gel diffusion against anti-Fc and reactions of partial identity with Fc in the same system (fig. 2b). Fraction 4 gave a reaction of identity with pFc′ ($\equiv C_H3$). Fraction 3 showed a single precipitin line with anti-C_H2 which fused with Fc; fraction 4 gave no reaction. These observations indicated that 3 and 4 were distinct components corresponding antigenically to C_H2 and C_H3, respectively.

A partial amino acid analysis of fraction 3 after a 20 hr acid hydrolysis is given in table 1. A striking similarity is apparent between the amino acid composition of fraction 3 and that of residues 223 to 334 (table 1) of the heavy chain sequence determined for protein Eu [13]. This latter sequence represents the N-terminal 111 residues of plasmin Fc and includes much of the 'hinge' region in addition to C_H2. In contrast a comparison of the amino acid compositions of fraction 3 and pFc′ showed large differences (table 1). The N-terminal residue of fraction 3 was shown to be threonine, using the dansyl-Edman method; the same as the parent Fc-

Table 1
Amino acid composition of fractions 3 and 4 isolated by column chromatography as shown in fig. 1.

	Fraction 3			Fraction 4			Fraction 3 obs. minus fraction 4 calc.[e]
	Obs.[a]	Calc.[b]	Diff.	Obs.[c]	Calc.[d]	Diff.	
Lys	8.3	8	0	6.8	7	0	1
His	3.7	4	0	3.3	3	0	1
Arg	3.0	3	0	2.4	3	0	0
Asp	10.4	10	0	11.0	11	0	1
Thr	8.8	8	1	7.7	8	0	1
Ser	7.1	6	1	11.9	15	3	8
Glu	11.9	11	1	14.7	15	0	3
Pro	12.6	13	0	11.2	10	1	3
Gly	4.8	4	1	5.9	6	0	2
Ala	3.4	3	0	2.8	3	0	0
Val	13.4	15	2	8.7	9	0	4
Met	1.1	1	0	0.9	2	1	1
Ile	2.0	2	0	1.6	2	0	0
Leu	8.0	8	0	8.1	4	4	4
Tyr	3.8	4	0	4.8	5	0	1
Phe	3.0	3	0	3.9	4	0	1
$GluNH_2$	6.0	–	–	0.0	–	–	–
Total differences (%)			6 (5.7%)			9 (8.4%)	31 (29%)

a) Residues/8 residues of leucine.
b) Calculated from composition of residues 223 to 334 of Eu sequence [13].
c) Residues/11 residues of aspartic acid.
d) Calculated from composition of residues 335 to 446 of Eu sequence [13].
e) These data have been included to stress the differences in composition between fraction 3 (putative C_H2) and C_H3.

fragment. The detection of glucosamine in fraction 3 was particularly interesting since the only point of attachment of carbohydrate in human γ-chain in asp-297 in the C_H2 region [13].

Preliminary physico-chemical studies on fraction 3 yielded a sedimentation coefficient of 2.4 S in 0.15 M NaCl, 10 mM Tris, pH 7.8 suggesting a molecular size near 22,000. Molecular weight determinations by sedimentation equilibrium after reduction and alkylation gave a value near 11,000.

For complement fixation tests proteins were aggregated by adsorption onto polystyrene latex particles [10]. Because of the differing affinities of IgG and the various fragments for polystyrene latex, different free protein concentrations were used in each test system in order to maintain a monolayer on the particles. The appropriate concentrations were determined for each protein by constructing adsorption isotherms as described by Oreskes and Singer [11]. Quantitative complement fixation tests were performed [12] and CH_{50} units estimated from the intercepts of von Krogh plots using data from the 20–80% hemolysis range for each concentration of protein tested. Controls with either polystyrene latex or protein alone showed no significant complement consumption, results are shown in table 2. The IgG, Fc and fraction 3 were all active in fixing complement in a dose dependent fashion, whereas fraction 4 and Fab showed no ability to react with complement in this system.

4. Discussion

Exposure of Fc (or rabbit IgG [6]) to acid pH appears to render the region between C_H2 and C_H3 susceptible to proteolysis. Conformational studies, using circular dichroism, showed that Fc undergoes a structural transition at acid pH which is fully reversible in less than 5 min after readjusting to pH 7.8 (to

Table 2
Complement fixing by IgG and its fragments adsorbed onto polystyrene latex (P.L.)

Protein	Total P.L. bound protein (μg)	Initial CH_{50} units	CH_{50} units consumed
IgG	15.0	20.9	20.9
	7.5	20.9	12.7
	3.7	20.9	10.6
	1.85	20.9	5.0
Fc	21.0	26.9	20.2
	15.0	26.9	15.0
	7.5	26.9	4.5
	5.0	26.9	3.9
	3.7	26.9	3.7
C_H2 (fraction 3)	30.0	13.0	13.0
	15.0	13.0	13.0
	7.5	13.0	3.5
	3.7	13.0	1.7
C_H3 (fraction 4)	15.0	11.0	0.0
	10.5	11.0	0.0
	7.5	11.0	0.27

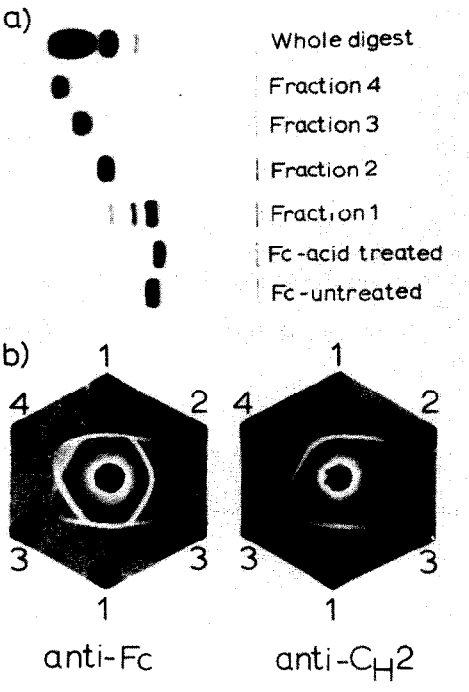

Fig. 2. a) Acid–urea starch gel electrophoresis, at pH 3.5, of the samples indicated. The cathode is on the left. b) Antigenic relationships between: 1, Fc'; 2, pFc' (prepared according to Turner and Bennich [3]); 3, Fraction 4 and 4, Fraction 3, using antisera to Fc and C_H2.

be published). This finding correlates with the time course of the loss of susceptibility to trypsin. Although the cleavage of Fc appears to be a complex process involving a number of intermediate species the evidence presented here indicates that fragments closely corresponding to C_H2 and C_H3 are produced in significant yields.

In formulating the compact domain model Edelman et al. [13] proposed that each domain had evolved to fulfil specific functions. Our data would suggest that C_H2 functions to trigger the complement system. This does not preclude other roles for C_H2 and the possible involvement of this region in other effector functions is being actively studied at this time. The differentiation of Fc with regard to complement activation and heterocytotropic activity seems clear since C_H2 does not participate in the passive cutaneous anaphylactic reaction and C_H3 is unreactive towards the complement system. The extent to which the complement fixing activities of intact IgG can be accounted for by isolated C_H2 remains to be fully evaluated. Previous work suggests that the complement binding site may occupy only a limited region of the C_H2 sequence: Utsumi [14] has shown that progressive degradation of rabbit Fc from the N-terminus results in the complete loss of complement fixing activity. Kehoe and Fougereau [10] have demonstrated low level residual complement fixing activity in a CNBr peptide derived from the C_H2 region of a murine IgG 2a myeloma protein. The technique of studying complement fixation by molecules aggregated on polystyrene latex needs further characterization. The possible dependence of the complement fixing activity of C_H2 on the integrity of the Fc region raises questions concerning the domains. Our desire to examine these putative interactions provided the impetus to develop the methods for the isolation of intact domains described here.

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