CONFORMATIONAL STUDIES ON SUBFRAGMENTS FROM THE Fc REGION OF HUMAN IMMUNOGLOBULIN G.*

K.J. Dorrington**, H. Bennich and M.W. Turner

Department of Biochemistry, University of Toronto
TORONTO, Canada, The Wallenberg Laboratory,
University of Uppsala, UPPSALA, Sweden, and
Department of Immunology, Institute of Child Health
University of London, LONDON, England.

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Summary: The circular dichroism (CD) spectrum of Fc fragment from Immunoglobulin G is dominated by a negative band at 217 nm. In contrast, pFc', a subfragment of Fc representing a dimer of the C_H3 domains of heavy chain, showed no minimum at this wavelength but rather at 224 nm. Marked differences were also apparent in the aromatic side chain CD region above 250 nm. A papain-produced fragment of Fc related to but smaller than pFc' showed CD properties quantitatively and qualitatively different from pFc'. Studies with a tryptic fragment of pFc' indicated that the conformational differences between pFc' and Fc' were primarily related to the proteolytic removal of a C-terminal tridecapeptide in the Fc' sequence relative to pFc'. A comparison of the CD spectra of pFc' with the constant region domain of light chain indicates that despite marked sequence homology between domains their conformations may be quite different.

INTRODUCTION: The 'compact-domain' model of Immunoglobulin G (IgG) is derived from evidence suggesting that the heavy (H) and light (L) chains are tightly folded into sequence homology regions of about 110 residues, each including a single intrachain disulphide bridge (1,2). There are two domains in the L chain and four in the H chain. The coincident suggestion that each domain has evolved to fulfil a specific biological function has stimulated attempts to isolate individual domains. The Fc region of IgG, which is composed of the two C-terminal homology regions (C_H2 and C_H3) of the two H chains, mediates a number of effector functions (e.g. complement activation, placental transfer, cytophylic activity). A subfragment of Fc, corresponding to the C_H3 domain (pFc') has been isolated from peptic digests of human (3,4) and rabbit (5) IgG. Other fragments of Fc, related to but smaller than pFc', may be isolated from papain digests of IgG (Fc') or trypsin digests of pFc' (6). The probable

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**To whom correspondence should be directed.

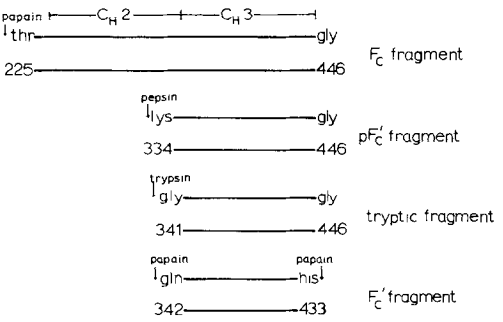

Figure 1. Schematic representation of the probable molecular location of the different Fc subfragments and their relationship to the C_H3 homology region. The numbering of residues is based on the Eu sequence (1).

molecular location of these fragments within Fc is shown in Figure 1.

In this study we have used circular dichroism (CD) to show the conformational uniqueness of the C_H3 domain and the sensitivity of its conformation to further limited digestion.

MATERIALS AND METHODS: Pooled human IgG was obtained from Kabi A.G. (Stockholm, Sweden) and a myeloma IgG from the serum of a patient with multiple myeloma (7). IgG was digested with papain according to the method of Porter (8). The Fab and Fc fragments, isolated by Sephadex G-150 chromatography, were separated by zone electrophoresis on cellulose. Plasmin Fc was prepared as described by Connell and Painter (9). pFc' fragments were prepared from pooled human IgG and the IgG 1 myeloma protein, and Fc' only from the pooled IgG, as previously described (3). Tryptic subfragments of pFc' were prepared from 4 hr digests (enzyme: substrate, 1:50) by Sephadex G-50 chromatography (6). Structural studies confirmed that the subfragments of Fc used in the present study had identical properties to those described earlier (3,6).

CD spectra were obtained with an ORD/CD-5 spectropolarimeter (Durrum/Japan Spectroscopic Co.) equipped with the SS-20 CD modification (7). Data is presented as mean residue ellipticity, $[\theta]$, in degrees cm^2./decimole using a mean residue weight of 110. Protein concentrations were determined spectrophotometrically at 280 nm, setting $E^{1\%}_{1cm} = 14.0$ in all cases.

RESULTS AND DISCUSSION: The CD spectra, between 210 and 320 nm, of papain Fc

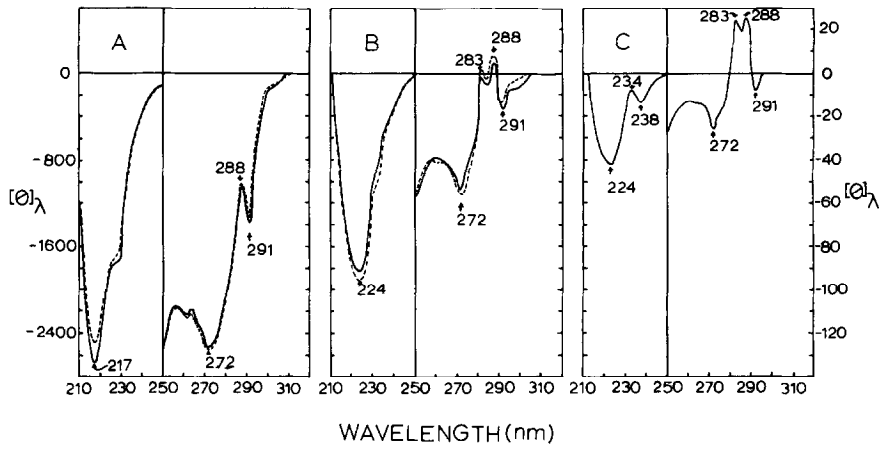

Figure 2. Circular dichroism spectra, between 210 and 320 nm, of: A. Fc fragments derived from a papain digest of pooled human IgG (——) and a plasmin digest of a human IgG 1 myeloma protein (----). B. pFc' fragment (——) and tryptic fragment of pFc' (----) from pooled human IgG. C. Fc' fragment derived from pooled human IgG. $[\theta]_\lambda$ is the mean residue ellipticity expressed in units of degrees. cm^2/decimole.

from pooled IgG and plasmin Fc from an IgG myeloma protein are shown in Figure 2A. As anticipated the spectra are essentially identical and similar to those published previously (10). Below 250 nm the spectrum is dominated by a negative band at 217 nm with clear evidence of another transition near 225 nm. The 217 nm band, frequently attributed to β-structure, is a characteristic feature of the CD spectra of intact IgG, H and L chains (7), and the variable and constant regions of L chain (11,12) as well as the Fab and Fc fragments. Overlapping bands due to aromatic side chains and cystine are apparent above 250 nm. The allocation of all these transitions is not possible, but the 272 nm band is probably due to tyrosine and the 291 and 288 nm transitions are almost certainly due to tryptophan (7).

The pFc' fragment, a dimeric structure representing the C_H3 domains of the two heavy chains, gives a CD spectrum quite different from Fc (Figure 2B). There is no evidence of a 217 nm minimum but rather a single negative band is present at 224 nm as noted previously (13). It seems probable that the shoulder at this wavelength in the CD spectrum of Fc is contributed by the C_H3 region. In the region above 250 nm the most striking change is the increased resolution of the tryptophan bands at 283, 288, 291 and 296 nm.

The putative tyrosine transition at 272 nm is still evident.

Perhaps the most striking observation reported here is the difference in CD between pFc' and Fc'. From Figure 1 it can be seen that Fc' is smaller than pFc' by only eight residues at the N-terminus and thirteen residues at the C-terminus. However these deletions cause a dramatic change in the optical activity (Figure 2C). The 224 nm band has half the intensity in Fc' as pFc'. In addition two transitions are resolved at 234 and 238 nm. Titration data suggest that the 234 nm band is due to tyrosine. Unfortunately the conformation of Fc' is sensitive to alkaline pH and a general unfolding of the fragment occurred before the 234 nm band could be fully titrated. These experiments did provide additional evidence however, that the 272 nm band in pFc' (and by analogy in Fc') was contributed by tyrosine residues. Above 250 nm the environment of tryptophan residues in Fc' show marked changes; the 283 and 288 nm bands are both positive (cf. pFc'), the 291 nm band is reduced in intensity and there is no evidence of a transition near 296 nm.

Under the conditions used in this investigation trypsin removes seven residues from the N-terminus of pFc', but leaves the C-terminus intact. The CD spectrum shows little change relative to that of pFc' (Figure 2B). This suggests that the marked differences in CD between pFc' and Fc' are due principally to the removal of the C-terminal tridecapeptide of each H chain. This region contains 2 tyrosines/chain which may account for the reduction of optical activity of Fc' in the spectral region associated with tyrosine transitions (e.g. near 270 nm).

The observations presented here may be relevant to a number of reports in the literature: Gm(a) and 'non-a' genetic antigens are demonstrable in pFc' and the tryptic fragment but not in Fc' (6). Yet available evidence suggests that amino acid residues correlating with these specificities are present in the Fc' fragment. It seems possible that the conformational changes accompanying the conversion of pFc' to Fc' are reflected in the loss of the antigenic expression of Gm(a) and 'non-a'. Charlwood and Utsumi (14) have

shown that the strength of the non-covalent association between the C_H3 domains increases with the progressive cleavage of rabbit Fc with papain. This suggests that the conformation of isolated pFc' may not truly reflect the contribution of C_H3 to the conformation of intact Fc. Clearly the isolation of the C_H2 domain must be achieved before the conformational relationships within Fc can be fully assessed.

It is interesting to note that although marked sequence homologies exist between C_H domains and C_L, this is not apparent when the CD spectra of C_H3 and C_L (11,12) are compared, suggesting conformational differences. These differences may form the basis of the functional differentiation within the immunoglobulin molecule.

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