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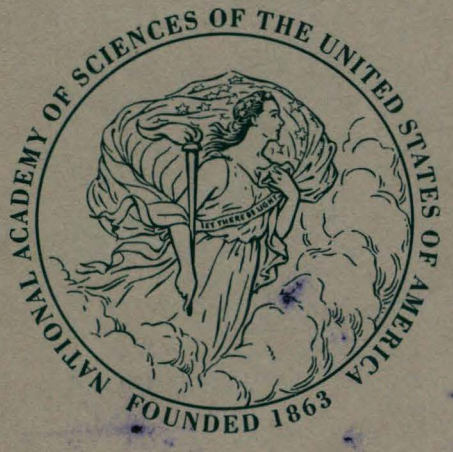

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*THE COVALENT STRUCTURE OF AN ENTIRE γG IMMUNOGLOBULIN MOLECULE**

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Abstract.—The complete amino acid sequence of a human γG1 immunoglobulin (Eu) has been determined and the arrangement of all of the disulfide bonds has been established. Comparison of the sequence with that of another myeloma protein (He) suggests that the variable regions of heavy and light chains are homologous and similar in length. The constant portion of the heavy chain contains three homology regions each of which is similar in size and homologous to the constant region of the light chain. Each variable region and each constant homology region contains one intrachain disulfide bond. The half-cystines participating in the interchain bonds are all clustered within a stretch of ten residues at the middle of the heavy chains.

These data support the hypothesis that immunoglobulins evolved by gene duplication after early divergence of V genes, which specified antigen-binding functions, and C genes, which specified other functions of antibody molecules. Each polypeptide chain may therefore be specified by two genes, V and C, which are fused to form a single gene (translocation hypothesis). The internal homologies and symmetry of the molecule suggest that homology regions may have similar three-dimensional structures each consisting of a compact domain which contributes to at least one active site (domain hypothesis). Both hypotheses are in accord with the linear regional differentiation of function in antibody molecules.

Antibodies or immunoglobulins can interact with a wide range of different antigenic determinants and, after specific binding to an antigen, they play a fundamental part in physiological functions of the immune response. The specificity of antigen binding depends ultimately upon amino acid sequences of the variable or V regions of antibody molecules. It is the diversity of these sequences which results in the range of specificities required for a selective immune response. In contrast, other regions of the antibody molecule have relatively constant sequences and are responsible for physiological functions. Like enzymes, these C regions appear to have evolved for a restricted set of interactions. This unusual picture of intramolecular differentiation has emerged from studies of the structure of immunoglobulins from different animal species.[1] To date, only portions of immunoglobulin molecules have been subjected to amino acid sequence determination.

We now report the amino acid sequence of an entire human γG1 immunoglobulin (molecular weight 150,000), the location of all disulfide bonds, the arrangement of light and heavy chains, and the length of the heavy chain V region.

Materials and Methods.—The isolation of the myeloma protein Eu[2] and the preparation of its CNBr fragments[3, 4] have been described. Similar methods were used for the isolation of the γG1 myeloma protein He and for the preparation of its CNBr fragments.

We have previously described the methods used for enzymatic digestion with trypsin, chymotrypsin, and pepsin, gel filtration, ion exchange chromatography, high voltage paper electrophoresis, determination of NH_2-terminal and COOH-terminal residues, amino acid analysis, and determination of amino acid sequences by the dansyl-Edman procedure.[4–8]

The positions of glutamine and asparagine were assigned[9] by determining the electrophoretic mobility of peptides and by amino acid analysis of the peptides after enzymatic hydrolysis. The half-cystinyl residues contributing to each intrachain disulfide bond were determined[10] using the diagonal electrophoresis method.[11]

Results.—The organization of the whole molecule is shown in Figure 1; an unequivocal proof of the arrangement of the two identical light chains and two identical heavy chains has already been given.[4] Each light chain is linked to its neighboring heavy chain by a disulfide bond between corresponding half-

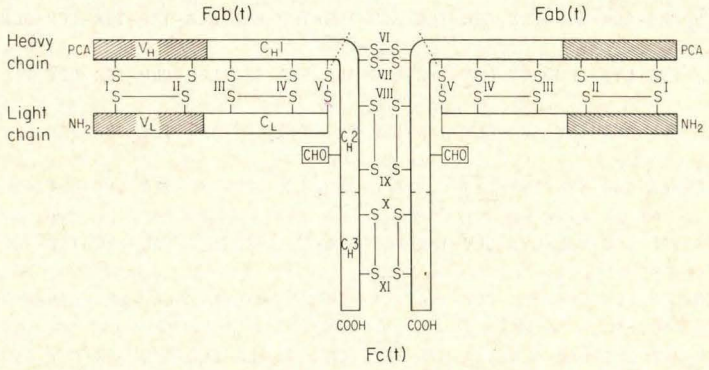

FIG. 1.—Over-all arrangement of chains and disulfide bonds of γG1 immunoglobulin Eu. Half-cystinyl residues are numbered I–XI; numbers I–V designate corresponding residues in light and heavy chains. PCA: pyrollidonecarboxylic acid. CHO: carbohydrate. "Fab(t)" and "Fc(t)" refer to fragments produced by trypsin, which cleaves the heavy chain as indicated by dashed lines above half-cystinyl residues VI. V_H, V_L: variable regions of heavy and light chains, C_L: constant region of light chain. C_H1, C_H2, C_H3: homology regions comprising C_H or constant region of heavy chain.

cystines V. Half-cystines VI and VII form bonds linking the half-molecules via the heavy chains. Trypsin cleaves the molecule at lysyl residue 222 to form two Fab(t) and one Fc(t) fragments.[2, 5]

There are several strikingly linear arrangements in the primary structure. From their amino termini to half-cystines V, the light and heavy chains can be aligned or put in register. The intrachain disulfide bonds are linearly and periodically disposed.[12, 13] In accord with the alignment of light and heavy chains, corresponding intrachain disulfide bonds are in similar positions and the disulfide loops are of approximately the same size.

Previous studies[7] have suggested that V regions of light and heavy chains have similar lengths and begin at the NH_2-termini; this will be confirmed below. The C_L region of the light chain has the same length as V_L, but the C_H region of the heavy chain is about three times as long. C_H may be divided into three

homologous regions of approximately equal length: C_H1, C_H2, and C_H3 (Fig. 1).

We have already reported the amino acid sequence of the first 87[7] and the last 224 residues[8] of the heavy chain as well as the partial sequence of the entire light chain.[6] The complete amino acid sequence of the light chain (214 residues) is shown in Figure 2. Positions of the half-cystinyl residues may be compared with Figure 1 and the methionyl residues may be correlated with previous studies on the CNBr fragments of Eu.[3, 4] The variable region extends through residue 108. In accord with other studies,[1] valine 191 is related to the Inv specificity.[2]

The complete sequence of the heavy chain (446 residues) is presented in Figure 3 which may be compared with Figure 2 for alignment with the light chain sequence. Isolation of a single glycopeptide[8] indicated that the polysaccharide

```
1                                                  10                                                  20
ASP-ILE-GLN-MET-THR-GLN-SER-PRO-SER-THR-LEU-SER-ALA-SER-VAL-GLY-ASP-ARG-VAL-THR-

                                                   30                                                  40
ILE-THR-[CYS]-ARG-ALA-SER-GLN-SER-ILE-ASN-THR-TRP-LEU-ALA-TRP-TYR-GLN-GLN-LYS-PRO-

                                                   50                                                  60
GLY-LYS-ALA-PRO-LYS-LEU-LEU-MET-TYR-LYS-ALA-SER-SER-LEU-GLU-SER-GLY-VAL-PRO-SER-

                                                   70                                                  80
ARG-PHE-ILE-GLY-SER-GLY-SER-GLY-THR-GLU-PHE-THR-LEU-THR-ILE-SER-SER-LEU-GLN-PRO-

                                                   90                                                  100
ASP-ASP-PHE-ALA-THR-TYR-TYR-[CYS]-GLN-GLN-TYR-ASN-SER-ASP-SER-LYS-MET-PHE-GLY-GLN-

                                                   110                                                 120
GLY-THR-LYS-VAL-GLU-VAL-LYS-GLY-THR-VAL-ALA-ALA-PRO-SER-VAL-PHE-ILE-PHE-PRO-PRO-

                                                   130                                                 140
SER-ASP-GLU-GLN-LEU-LYS-SER-GLY-THR-ALA-SER-VAL-VAL-[CYS]-LEU-LEU-ASN-ASN-PHE-TYR-

                                                   150                                                 160
PRO-ARG-GLU-ALA-LYS-VAL-GLN-TRP-LYS-VAL-ASP-ASN-ALA-LEU-GLN-SER-GLY-ASN-SER-GLN-

                                                   170                                                 180
GLU-SER-VAL-THR-GLU-GLN-ASP-SER-LYS-ASP-SER-THR-TYR-SER-LEU-SER-SER-THR-LEU-THR-

                                                   190                                                 200
LEU-SER-LYS-ALA-ASP-TYR-GLU-LYS-HIS-LYS-VAL-TYR-ALA-[CYS]-GLU-VAL-THR-HIS-GLN-GLY-

                                                   210                 214
LEU-SER-SER-PRO-VAL-THR-LYS-SER-PHE-ASN-ARG-GLY-GLU-[CYS]
```

FIG. 2.—Complete amino acid sequence of the Eu light chain. Half-cystinyl residues are in boxes and methionyl residues are underlined.

portion of the molecule is attached at Asx residue 297.[14] In a previous study[8] we have suggested that glutamyl residue 356 and methionyl residue 358 may be associated with Gm 1 specificities. The sequence of Eu (Gm 4+) between residues 211–252 can be compared with the partial sequence of immunoglobulin Daw[17] (Gm 4−). The presence of arginine in position 214 of Eu and lysine in a comparable position of Daw may be associated with their Gm 4 specificities.[18]

Of particular significance is the determination of the point at which V_H ends and C_H begins. A CNBr fragment comparable to fragment H_4 was isolated from myeloma protein He which has the same Gm specificity as protein Eu. The sequence of the amino terminal portion of the CNBr fragment from He differed from that of the H_4 fragment from Eu.[21]

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