HOW DID EDELMAN AND PORTER WIN THE NOBEL PRIZE

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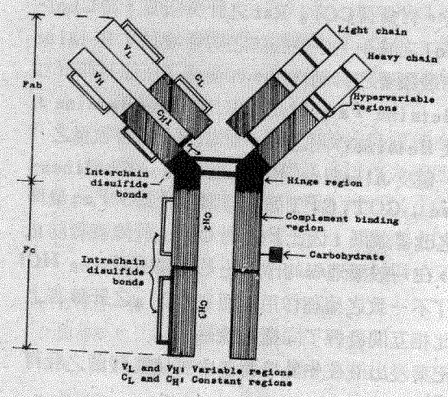
Jenner started clinical immunology with vaccination against smallpox in 1798. In recent 15 years there have been dramatic developments in molecular immunology. Edelman of Rockefeller University established that antibody molecule is made up of four polypeptide chains. Porter of St. Mary's Hospital Medical School in London digested IgG, with a proteolytic enzyme, papain, into three fragments; one Fc and two Fab. Nobel Prize of Medicine was awarded to them in 1972.

There are well over 100 kinds of proteins in circulating serum. When serum is electrophoresed, these proteins can be separated into five groups; albumin and four globulins, namely alpha-1, alpha-2, beta and gamma. Most antibodies are electrophoretically gamma and beta globulins. Usually antibodies are very heterogeneous. However the "abnormal" paraprotein made by monoclonal myeloma cells in large amount is homogeneous. The structural studies of immunoglobulins are usually carried out with these myeloma pro-

It will take time and place to explain how Edelman and porter formulated the basic structure of IgG starting with their original experiments. Here I show first the topology and functional architecture of IgG, summerized by Capra of Mt. Sinai School of Medicine in New York, with slight modification by the author and explain how they did with this molecule(Fig.1).

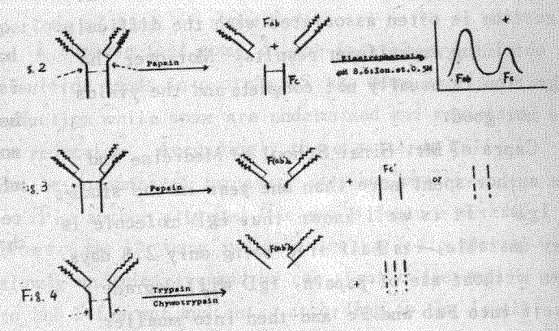
Figure 1. Topology and functional architecture of the gamma-G molecule.



In the mid-1950's, Porter used papain to digest IgG molecule and he got two fragments of Fab and one of Fc (Fig. 2)
The Fab fragments retained the mono-valent antibody combining site each and the Fc fragment was easily crystallized. The two Fab fragment produced by papain digestion are identical to one another. Each consists of a light chain and half of a heavy chain. It does not crystallize but it retains the antigen binding activity. It cannot link antigens together to form precipitation or agglutination, because it is mono-valent. The Fc fragment consists of two half heavy chains, is inactive in sense that it does not combine with antigen. On the other hand, Fc can crystallize, and is essential for certain biological activities of immunoglobulin, such as complement fixation, skin fixation, to cross placenta, to regulate immunoglobulin catabolism and others.

Now let us consider two problems. First, if we use other proteolytic enzymes, such as trypsin, chymotrypsin and pepsin, instead of papain, what will happen ? Secondly if we digest other immunoglobulins with the same enzyme, papain, what we will get ?

Many distinguished molecular immunologists have tried to use various enzymes to digest immunoglobulins. The phenomenon of specificity is one of the most characteristic properties of the proteolytic enzyme, just like that in antigen-antibody reaction. Usually before digesting a protein with a particular enzyme, we have to know not only the specificity of the enzyme but also some outlines of the chemical structure and physical properties of the protein, and so we can postulate how many fragments we will get. When Porter digested IgG with papain there was no much information about the structure of IgG. Probably before trying papain, he had also tried other enzymes to digest it, but without good results. He was lucky in the sense that papain hit the hinge region of IgG and the resultant subunits, Fab and Fc, with the same molecular size, could be separated each other easily with the use of electrophoresis or ion-exchange chromatography (Fig. 2), and only the Fab subunit retaind the antibody activity.

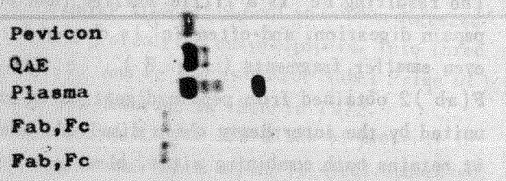


With the use of pepsin, the flexible hinge region is also cleaved, but the labile spot digested is usually a little further from N-terminal, beyond the inter-heavy chain disulfide bond. The resulting Fc' is a little smaller than Fc of papain digestion, and often Fc' is chewed into even smaller fragments (Fig. 3)
F(ab')2 obtained from pepsin digestion is still united by the inter-heavy chain disulfide bond and it retains both combining sites, bi-valent. If trypsin or chymotrypsin is used, Fc' fragment is always chewed into small fragments, leaving only Fab or F(ab')2 as the main product(Fig. 4).

Each myeloma protein has its own specific physicochemical properties, such as solubility, viscosity, chemical stability, reactivity with other proteins, three-dimensional conformation, and primary amino acid sequence. Accordingly an enzyme can spilit a particular myeloma protein at a certain point; the same enzyme may not be

able to spilit another myeloma protein at the same point. It has not been possible to obtain Fc together with Fab or F(ab')2 fragments, from the human IgA myeloma protein with the enzymatic digestion. Generally speaking the hinge region located at the middle portion of two heavy chain is flexible and more exposed. The hinge region is labile to enzymatic digestion. However the digestion is often associated with the difficulty in obtaining an uniform results. Moreover the digestion is usually not complete and the yields are not good.

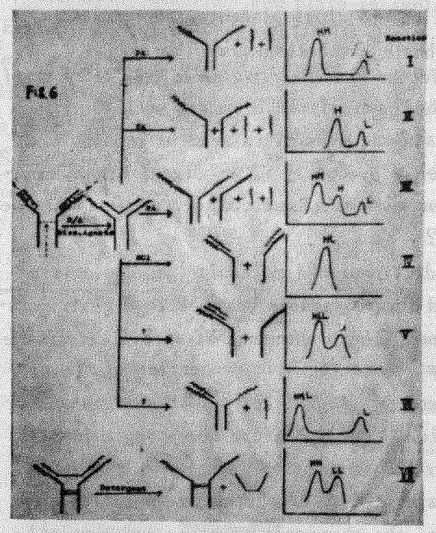
Capra of Mt. Sinai School of Medicine and the author spent more than one year in the studies of IgD. It is well known that IgD molecule is very unstable, its half life being only 2.8 days. Even without use of papain, IgD will degrade itself into Fab and Fc and then into smaller fragment, probably due to plasmin or other proteolytic enzymes present in serum(Fig. 5) It was so unstable that we could not purify any one of the segments for the sequencial study.



Now we turn to Edelman's work. He used a reducing agent (beta-mercaptoethanol) which splits disulfide bonds linking heavy and light chains, and a dissociating agent (propionic acid) which separate these polypeptide chains*.

By treating an IgGl(Eu) with these substances he demonstrated the multichain structure of the immunoglobulins in 1959 and in 1969 he publishec its entire primary structure.

Before Edelman's work, many biochemists had used reduction with alkyation to treat proteins. Immunoglobulin chains are linked together by covalent disulfide bonds and non-covalent ineractions, such as hydrophobic bonds and electrostatic forces. It is easy to split disulfide bonds with reduction and alkylation, however it is necessary to use dissociating agens to separate heavy chain from light chain, combating with the non-covalent bonds operating between the two types of chains. With IM propionic acid, Edelman successfully separated the heavy chain from light chain (Fig. 6, Reactions I,II and III)



Nisonoff of University of Illinois used hydrochloric acid, its PH was Kept 2.6, exactly the same with PH of IM propionic acid, but he could

* In this short discussion, we only deal with imcomplete reduction which only splits inter-chain bonds but not intra-chain bonds.

only get one type of subunit, half molecule, consisting one heavy and one light chain(Reaction IV).

In 1968, Grey at Kunkel's laboratory, showed that proteins of IgA_2 subclass have no H-L disulfide bonds and instead they have L-L disulfide bonds. Accordingly if you treat IgA_2 proteins with detergents, such as urea or guanidine HCl, you can separate heavy chain from light chain without reduction with alkylation (Reaction VII).

Usually after reduction with alkylation heavy and light chains are still held together with non-covalent forces. Why HCl dissociate immunoglobulin into half molecule subunit, and propionic (and also acetic acid) dissociate the same into heavy and light chain ? Nisonoff explained himself that HCl lacks the weak detargent action of PA. I suppose other factors, such as difference of the physicochemical properpies of the protins, are also involved. There are several possibilities of combination of the four chains in separating them. In practice with the use of PA only theee patterns are encountered, Reactions I,II and III.

Reaction I shows that all heavy chains are eluted from G-100 Sephadex colomn in a dimer form, and Reaction II shows that all heavy chains are eluted in a monomer form, all completely dissociated. Reaction III is the combination of I and II, portion of heavy chains are in dimer form. It is generally accepted that the non-covalent forces between the two heavy chains in the same molecule is strong but that operates between heavy chavy chains of different molecules is neglisible in IM PA. Accordingly there will be no heavy chain trimer or heavy chain aggregates. It is also accepted that the non-covalent forces between the two heavy chains in the molecule is strongest in Fc segment, but these forces between two light chains and between heavy-light chain are neglisible in propionic acid solution. Accordingly Reactions V, and VI are not encountered Edelman's work is so great in the sense that all immunoglobulins can be treated in this way with almost 100% recoveries.

Kochwa and Capra reduced and alkylated IgE (PS) and separated heavy chain and light chain with different patterns according to the varying periods of time of reduction.

I suppose that some of the inter-heavy chain disulfide bonds in protein PS are more labile to reduction while some are undermined and resistant to reduction. Accordingly these patterns will not be encountered for every immunoglobulin.

The author attended Edelman's lecture several times. He is young and good looking. He speaks slowly and clearly, and he can speak differently to the different audients, according to the levels of understanding by the audients. He never said a word of no importance, but when he inserted a word of joke, everybody laughed; indeed he is a genus in teaching! He worked day and night and also in holidays. In United States, they have two holidays a week, but the holidays are for the people other than doctors. They say that Edelman's wife never complained of her loneliness. They also say that Edelman is very smart, but I think everybody at the Lockfeller University is very smart too. From his colleagues I heard that Edelman is active, confident and sometimes aggressive. In his earlier career he talked very much and tried to publish many papers, but his instructor, Dr. Kunkel, stopped him, advising him to repeat his studies. He did not obey his instructor, and once he was almost fired.

To win the Nobel Prize, I think, you have to be lucky, smart, eloquent, diligent, aggressive and to have a good wife. You have also to be a basic sience man; if you want to be a clinician, or if you are already so, please foreget the Prize !

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