A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-12; Unit II)

Antibody

Gross Structure

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DEFINITION:

- An antibody or immunoglobulin (Ig) is a glycoprotein that is made by plasma cells in response to an antigen and can recognize and bind to the antigen that caused its production.
- Antibodies bind antigen with a high degree of specificity and affinity. Antibodies recognize a variety of three-dimensional shapes (amino acids, lipids, carbohydrates, etc.).
- Antibodies have more than one antigen combining site Some bivalent Ab molecules can combine to form multimeric Abs that have upto 10 combining sites

CLASSSICAL EXPERIMENT OF ANTIBODY

The first evidence that antibody were contain in particular serum protein fraction proposed by A. Tiselius & E. A. Kabat in 1939

They immunized rabbits with the protein ovalbumin (the albumin of white egg).

Then divided the immunized rabbits' serum into two aliquots.

Electrophoresis of one serum aliquots revealed four peaks corresponding to albumin & the alpha ,beta & gamma globulin.

The other serum aliquots was reacted with ovalbumin & the precipitate was removed.

The remaining serum proteins ,which not react with Ag, were then electrophoresed.

A comparison of the electrophoretic profile of these two serum aliquots revealed.

there was a significant drop in the gamma globulin peak in the aliquot that had been reacted with Ag.

Thus the gamma globulin fraction was identified as containing serum Ab.

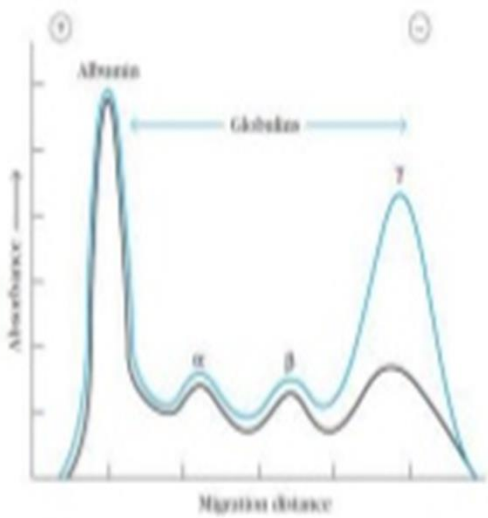
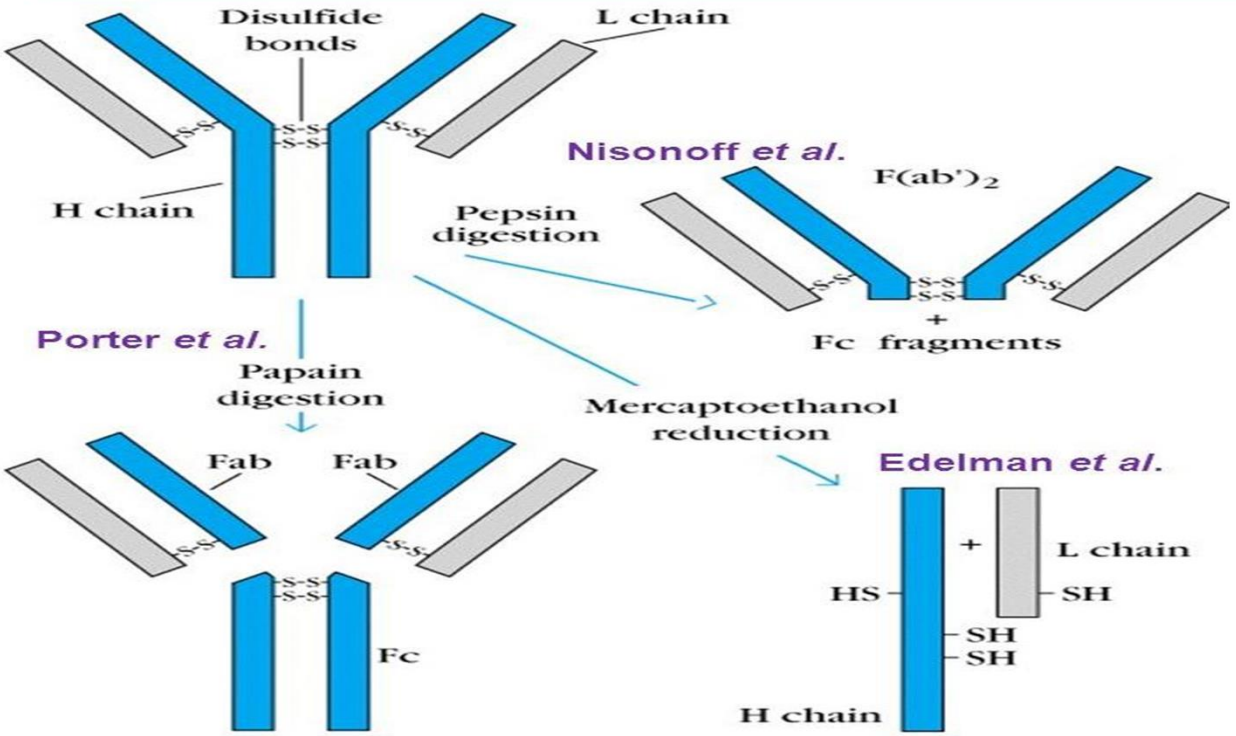


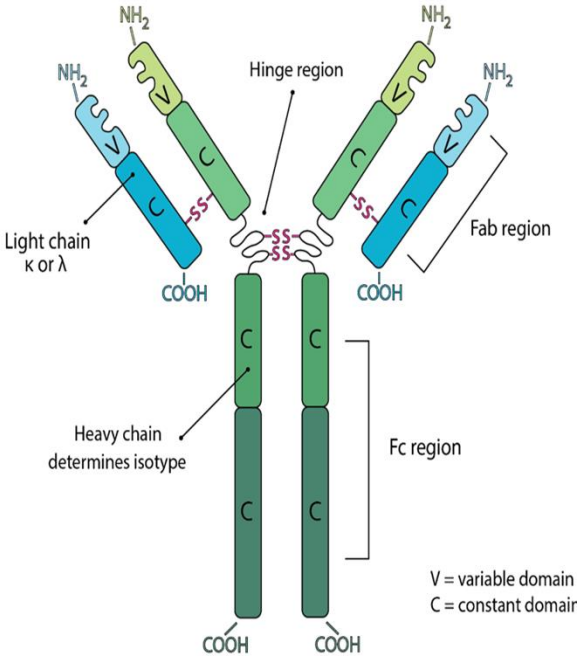
FIGURE 1

Experimental demonstration that most antibodies are in the γ-globulin fraction of serum proteins. After rabbits were immunized with ovalbumin (OVA), their antisera were pooled and electrophoresed, which separated the serum proteins according to their electric charge and mass. The blue line shows the electrophoretic pattern of untreated antiserum. The black line shows the pattern of antiserum that was first incubated with OVA to remove anti-OVA antibody and then subjected to electrophoresis. [Adapted from A. Tiselius and E. A. Kabat, 1939, Journal of Experimental Medicine 69:119, with copyright permission of the Rockefeller University Press.]

- By the 1960s, Gerald Edelman at Rockefeller University in New York and Rodney Porter at the University of Oxford, England worked out the structure and complete amino acid sequence of the antibody, IgG.

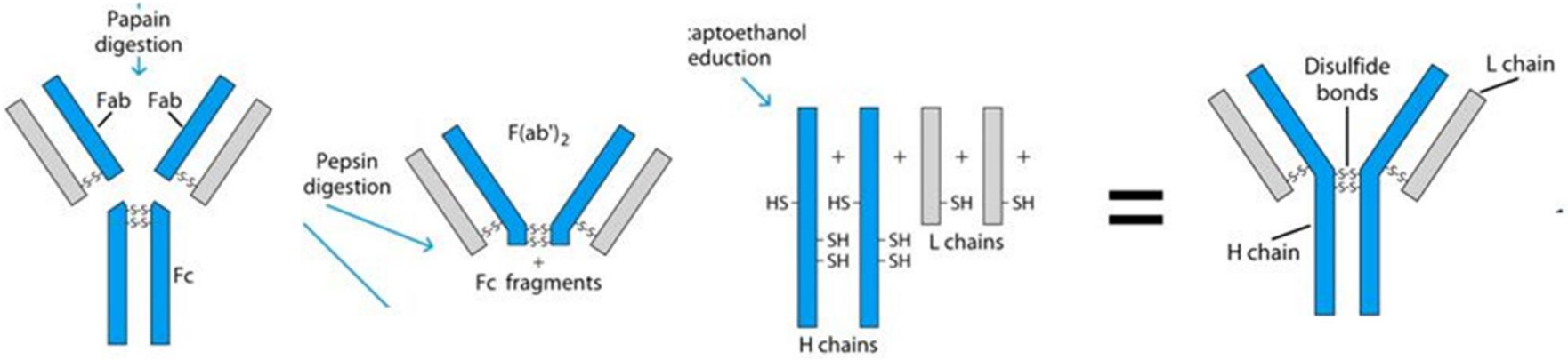
- In 1972, Gerald Edelman and Rodney Porter were shared the Nobel Prize in Physiology or Medicine "for their discoveries concerning the chemical structure of antibodies".





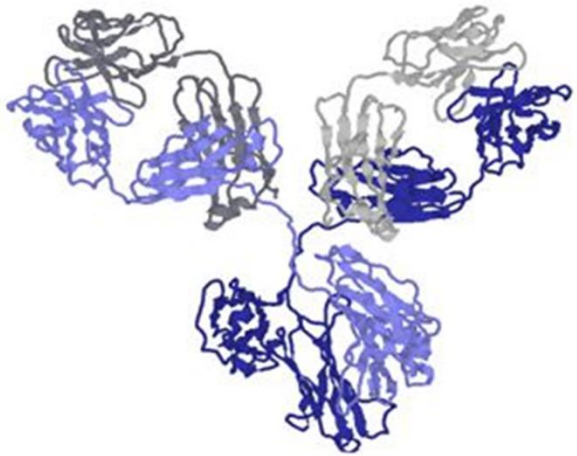
Elucidation of the basic structure of the immunoglobulin (IgG) molecule

- Edelman and Porter used ultracentrifugation and found that the γ-globulin fraction had a MW of ~150,000
- Papain digestion produced 2 identical fragments (MW 45,000) called Fab that retained Ag-binding and 1 Fc fragment (MW 50,000)
- Pepsin digestion resulted in 1 Fab-like fragment (MW 100,00) called F(ab')2 that retained Ag-binding
- Mercaptoethanol reduction and alkylation (cleaves S-S bonds) revealed that the 150,000 molecule was composed of 2 identical 50,000 MW polypeptide chains (H-chains) and 2 identical 25,000 MW chains (L-chains)

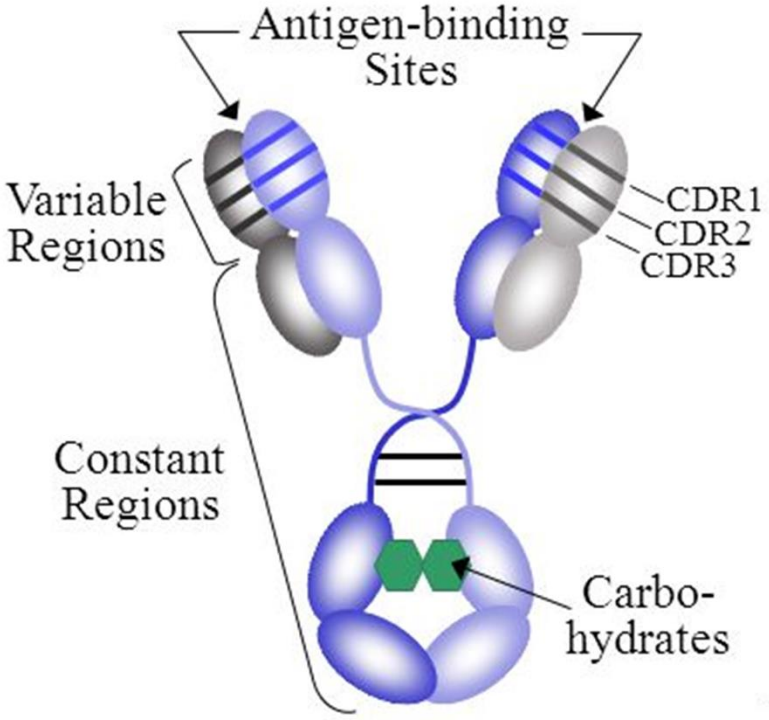


Antibody Structure

- Antibodies, or immunoglobulin (Ig) molecules, are composed of four chains, two light and two heavy
- The structure of Ig molecules can be divided into a constant region, in which the amino acid sequence is largely conserved, and a variable region, where the amino acid sequence for different Ig molecules has considerably more variation
- Within the variable region, there are three hypervariable regions, referred to as complementarity determining regions (CDR1-3), as they are located at the binding sites of the antibody molecule



RasMol image of IgG molecule, PDB code 1IGT, Harris, L. J., Skaletsky, E., and McPherson, A. (1998) *J. Mol. Biol.* 275, 861-872.



Antibodies structure

Each antibody consists of four polypeptides– **two heavy chains and two light chains** joined to form a "Y" shaped molecule.

This variable region, composed of 110-130 amino acids, give the antibody its specificity for binding antigen.

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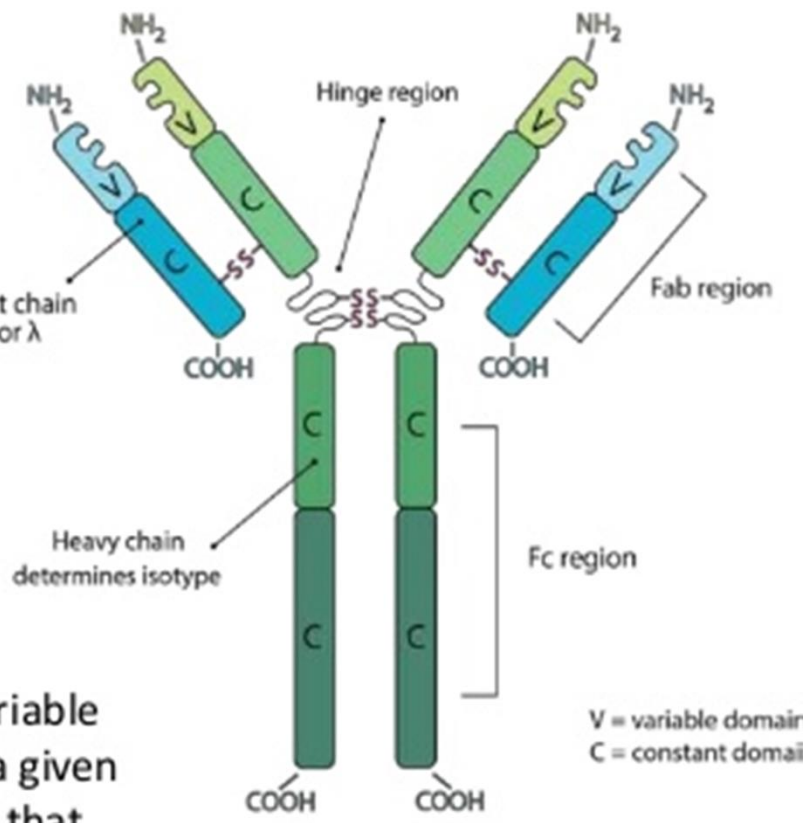
The variable region is further subdivided into **hyper variable (HV) and framework (FR) regions.** Hyper variable regions have a high ratio of different amino acids in a given position, relative to the most common amino acid in that position. Within light and heavy chains, three hyper variable regions exist – HV 1, 2 and 3. Four FR regions which have more stable amino acids sequences separate the HV regions.



H chains

- 5 classes
- Structurally and antigenically distinct
- Each designated by Greek letter
- 5 classes of Ig (IgG, IgA, IgM, IgD and IgE) classified based on AA sequence of heavy chains

- **L chains**
- 2 types
- Kappa (κ) and lambda (λ) named after Korngold and Lapari
- In humans, L chains, 60% kappa and 40% lambda
- Both light chains of Ab molecule should be same type, either κ or λ, never both

classification of Antibodies

The simplest and most abundant immunoglobulin are in size and monomers, but they can also assume some differences arrangement.

The five classes of Igs are designated

The five classes of antibodies are following

1. Ig G,(Immunoglobulin G)
2. Ig M,(immunoglobulin M)
3. Ig A,(Immunoglobulin A)
4. Ig D,(Immunoglobulin D)
5. Ig E.(Immunoglobulin E)

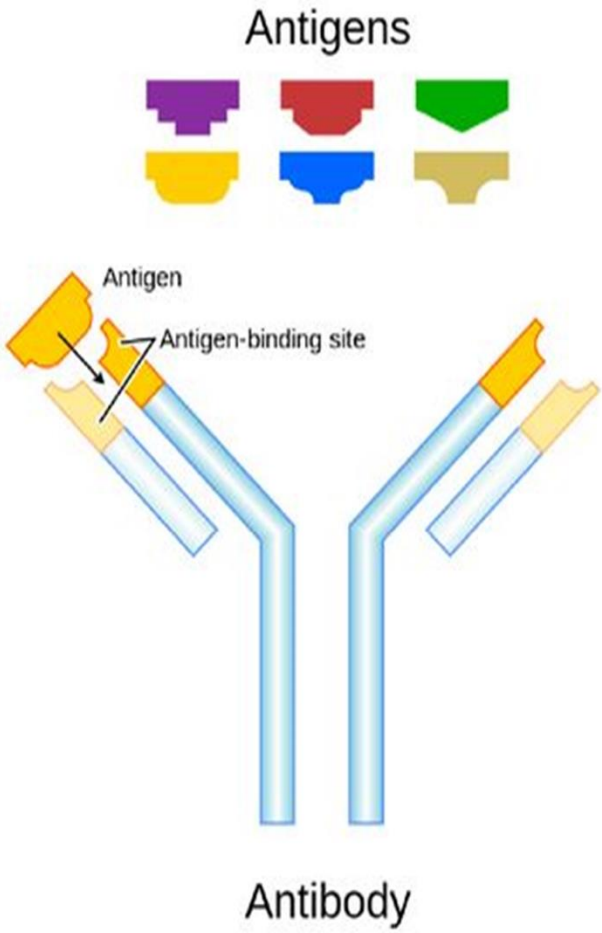
These are classified by the kind of heavy chain found in the molecule.

Properties of immunoglobulins/antibodies:

	IgG	IgA	IgM	IgD	IgE
1. Serum conc. (%)	85	5-15	5-10	<1	<1
2. Mol. Wt.	160,000	170,000 & 385,000	960,000	184,000	188,105
3.Sed. coeff.	7S	7S	19S	7S	8S
4.Heavy chain class	Gamma	Alpha	Mu	Delta	Epsilon
5.Light chain	K & L	K & L	K & L	K & L	K & L
6. Valency	2	2 or multiple of 2	5 (10)	2	2
7.No of basic 4-polypeptide chains	Monomer	Monomeric(serum) or dimeric (secretory)	Pentamer	Monomer	Monomer

Diversity

- Antibodies come in millions of different amino acid sequences and are the most diverse proteins known.
- Because the amino acid sequence differs in the arms of various antibody molecules, each different antibody can bind specifically to one unique epitope.
- Thus, the arms of an antibody molecule confer the specificity of responses that a host can mount against antigens.
- The stem region of an antibody molecule bear its biological activity and defines whether the response against a particular antigen will lead to complement-mediated lysis, enhanced phagocytosis, or (in some cases) allergy. These activities start once antibodies bind to antigen.



	Polyclonal	Monoclonal	Single Domain
Advantages	• Inexpensive to produce • Short turnaround time • Broad utility • Requires ~1mg of antigen • Multiple host species	• Unlimited supply • Lot-to-lot consistency • High specificity	• Unlimited supply • Lot-to-lot consistency • High specificity • High stability • Low cost large scale production
Disadvantages	• Limited availability • Lot-to-lot variation • Difficult to reproduce	• Cost of development • 4-6 month development • Requires 5+ mg of antigen	• Cost of development
When to Choose	• Need qualitative detection • Need antibody quickly • Need low cost antibody • Need high affinity	• Need quantitative detection • Need consistent antibody • Need high specificity	• Need quantitative detection • Need consistent antibody • Need large quantity • Need high specificity & affinity

Acknowledgement and Suggested Readings:

1. Kuby Immunology; Sixth Edition; Kindt, Goldsby and Osborne; W. H. Freeman and Company
2. Fundamental Immunology; 5th edition; William E., Md. Paul (Editor) ; Lippincott Williams & Wilkins Publishers
3. Roitt's Essential Immunology; Tenth Edition; Roitt and Delves; Blackwell Science
4. Cellular and Molecular Immunology; 6th Edition; Abbas, Lichtman and Pillai; Saunders Elsevier

Thanks