Immunochemical assays

Seminar No. 7

- Chapter 23 -

Immunochemical assays are based on antibody-antigen *in vitro* interaction

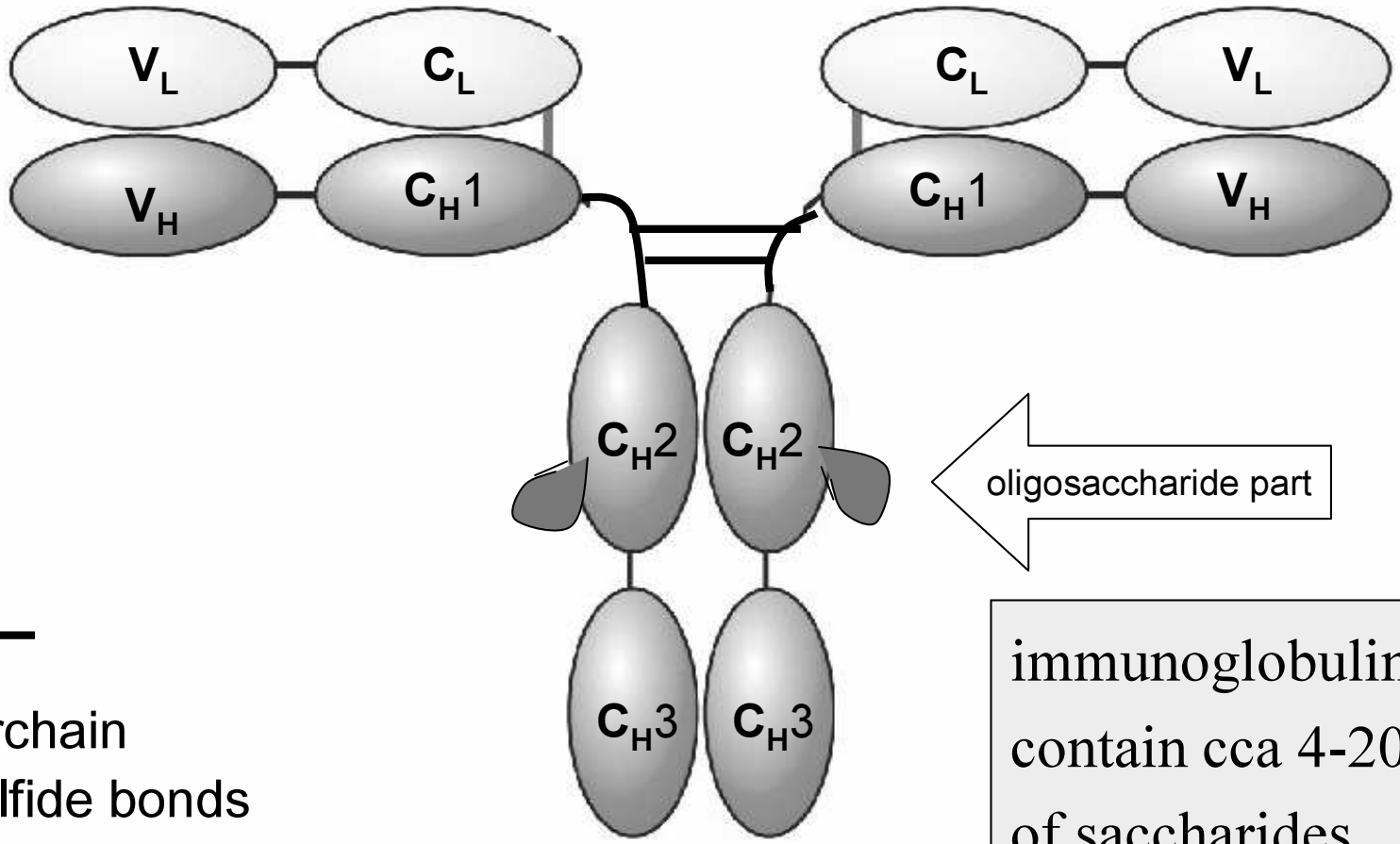
Q.

What is antibody?

Antibody

- a defense protein synthesized by the immune system
- immunoglobulins are plasma proteins produced by lymphocytes in response to some external molecules
- they are capable to bound specifically to antigen

The scheme of immunoglobulin molecule



interchain
disulfide bonds

immunoglobulins
contain cca 4-20 %
of saccharides

Q.

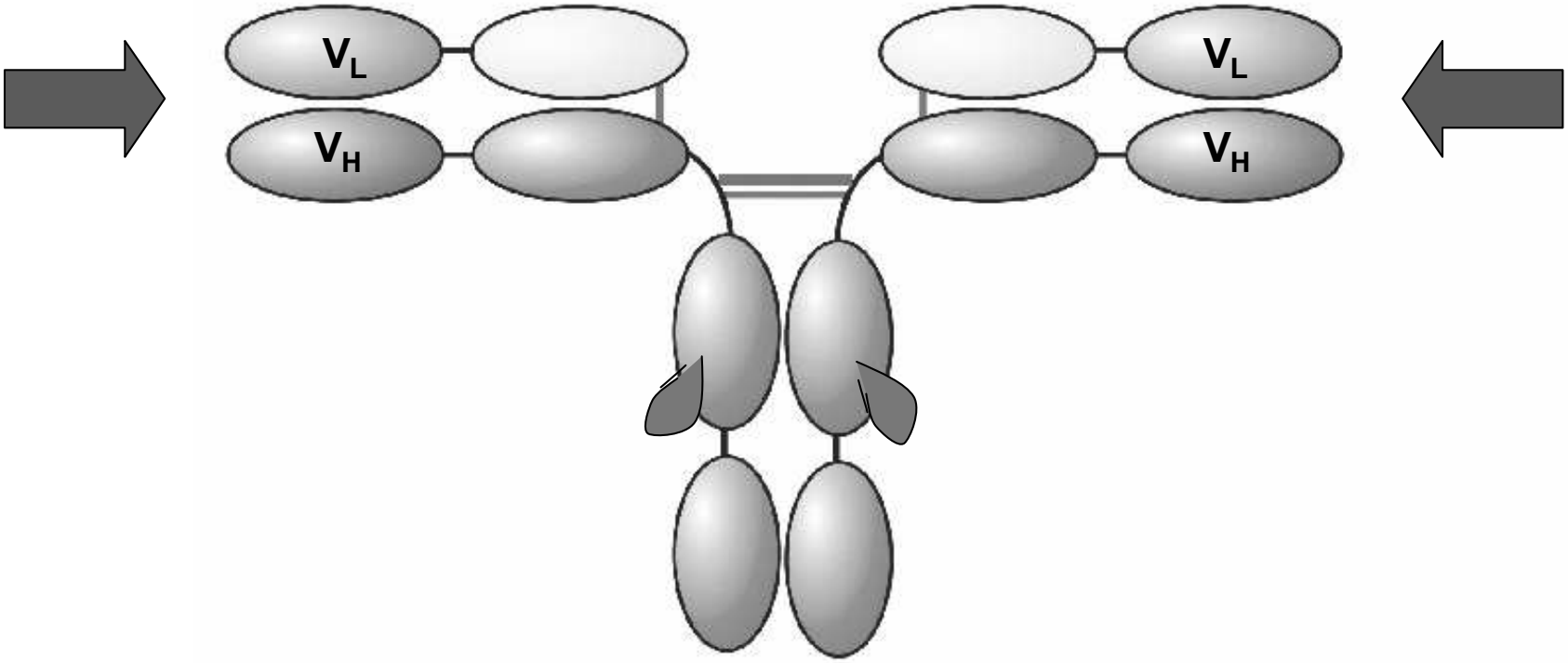
How many chains are there in immunoglubulin molecule?

Immunoglobulin molecule

- Y-shaped molecule
- two heavy chains (H) + two light chains (L)
- the chains are composed from series of domains
- each chain contains variable (V) and constant (C) domains
- each domain is closed by a single -S-S- bridge

Domain is a distinct structural unit of a polypeptide.
Domains may have separate functions and may fold as independent, compact units.

Variable domains of H/L chains contain binding sites for antigen



Q.

What is antigen?

Antigen = antibody generating

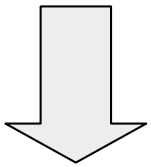
- a foreign macromolecule that provokes the formation of antibodies
- proteins, glycoproteins, lipoproteins, polysaccharides ...
- may be bound on the surface of cells or particles (viruses, bacteria, pollens, tissues from another individual)

- **hapten** (= incomplete antigen), small molecule
- can become antigen when bound to protein

Interaction of antigen (Ag) with antibody (Ab)

- highly specific
- based on non-covalent interactions (mainly hydrophobic)
- Ag + Ab $\rightarrow$ immune complex $\rightarrow$ other changes (in vivo, in vitro)

- in vivo: phagocytosis
- in vitro: **precipitate** (insoluble in water)



determination of precipitate quality and/or quantity

Immunochemical assays exhibit high specifity and sensitivity

Method	Feature
Immunoprecipitation	precipitation in solution or gel medium
Immunoelectrophoresis	Ab/Ag migration in electrical field
Radioimmunoassay	Ab/Ag labelled with radioisotope
Enzyme immunoassay	Ab/Ag labelled with enzyme

Immunoturbidimetry (p. 10)

- turbid = not transparent
- absorbance of light in colloid solution is measured
- $A \sim c$ (of immune complex)

Immunonephelometry (p. 10)

- nepheloid = turbid
- intensity of scattered light is measured
- $I \sim c$ (of immune complex)

Single radial immunodiffusion

- **only one reactant diffuses**
- Ag is applied into a hole in gel medium
- gel is impregnated with antibody
- Ag diffuses in all directions (360°)
- a circular immunoprecipitate is produced

Double immunodiffusion

both reactants (Ag, Ab) diffuse in all directions

The proof of Ag identity

- central well contains Ab, six Ag samples are places around
- if the same antigens are in opposite wells $\Rightarrow$ precipitation lines fuse (**identity**)
- if antigens are completely different $\Rightarrow$ precipitation lines cross each other (**non-identity**)
- if two antigens are related but not identical $\Rightarrow$ partially crossed lines (**partial identity**)

Immunoelectrophoresis of Ag mixture

- scheme on p. 139
- Ag mixture is applied into a hole in agarose gel
- simple electrophoretic separation afforded 5 fractions
- then **antiserum** (= mixture of antibodies) is applied into side troughs
- antibodies and separated antigens diffuse
- the result is a typical pattern of **several precipitation lines**

Rocket immunoelpho

- samples of Ag move through the gel with antibody
- precipitations are of rocket shapes (bec. of acceleration by electric field)
- for a given Ab concentration there is a linear relationship between the height of rocket and the Ag concentration

Crossed immunoelpho

- a more sensitive modification
- produces higher resolution
- 1st separation: elpho
- 2nd separatation: elpho at 90° angle to the 1st run through gel containing polyvalent antibody

Enzyme immunoassays

- Ab or Ag/hapten is labelled with covalently attached enzyme
- **peroxidase** (isolated from horseradish) catalyzes reduction of H_2O_2 or organic peroxides
- **ALP** (alkaline phosphatase, isolated from *Escherichia coli*) catalyzes the hydrolysis of synthetic alkyl phosphates

Homogenous EIA

- free hapten and enzyme-labelled hapten compete for binding sites in antibody
- enzyme in immunocomplex **is not active**, access to active site is blocked
- activity of free enzyme-hapten conjugate (= quantity of product) is determined
- the amount of product is directly proportional to the amount of hapten in sample

ELISA methods

Enzyme Linked Immunosorbent Assay

Competitive ELISA

- antibody is bound to solid phase (sorbent)
- antigen to be analyzed (Ag) and Ag-E conjugate compete for binding sites in antibody
- enzyme in immunocomplex **is active** and catalyzes the conversion of substrate to product
- the amount of product is indirectly proportional to the amount of Ag in sample