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VMC 607: Vaccinology

Topic: Methods of inactivation of pathogen

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Inactivated whole-cell vaccines

- Inactivated vaccines are made from microorganisms (viruses, bacteria, other) that have been killed through physical or chemical processes.
- These killed organisms cannot cause disease.
- They are no longer capable of replication in the host
- For effectiveness they contain much more antigen than live vaccines

Immune response

- Inactivated whole-cell vaccines may not always induce an immune response and the response may not be long lived.
- Several doses of inactivated whole-cell vaccines may be required to evoke a sufficient immune response.

IMMUNE RESPONSE

- May not always induce an immune response at first dose.
- Response may not be long-lived, requiring several doses of vaccine.
- Less strong immune response compared to live vaccines

SAFETY AND STABILITY

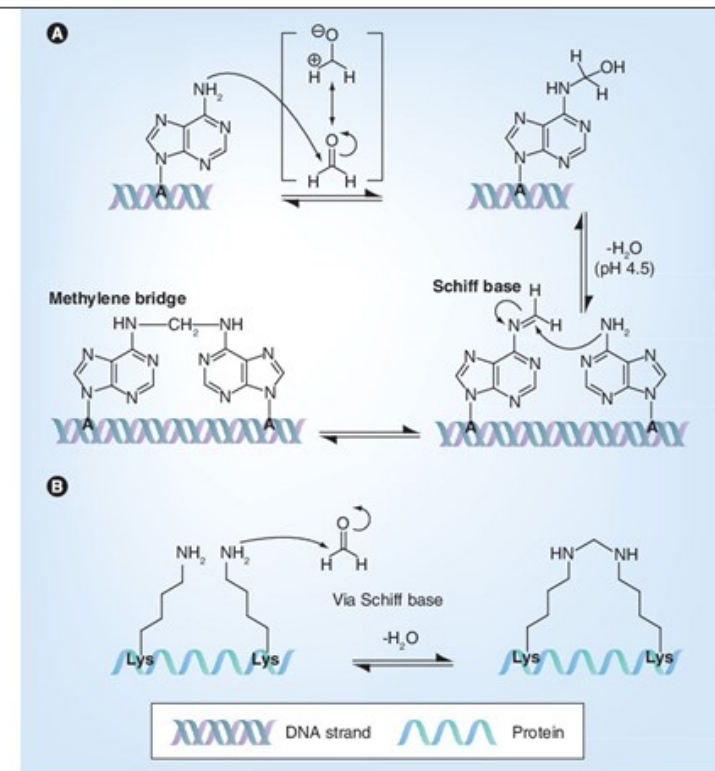
- **No live component**
 - no risk of inducing the disease.
- **Excellent stability profile**

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- Inactivated process is critically important to maintain the structure of epitopes on surface antigens during inactivation.
 - Excessive heat inactivation cause denaturation of protein therefore the epitope depend on structure of protein are altered or damaged.
 - Successful methods to kill the pathogen relies on chemicals such as
 - Formaldehyde
 - Phenol
 - β -propiolactone (BPL)
 - Binary ethylenimine (BEI)

Reaction mechanism of formaldehyde with either DNA/RNA or amino acids.

Formaldehyde when diluted in water, has an electron deficient central carbon atom and is therefore electrophilic, as illustrated through one of the resonance forms.

As a consequence, a nucleophile, such as a nonprotonated amino group, can attack the central carbonyl carbon- this chemical reaction is called a nucleophilic addition.



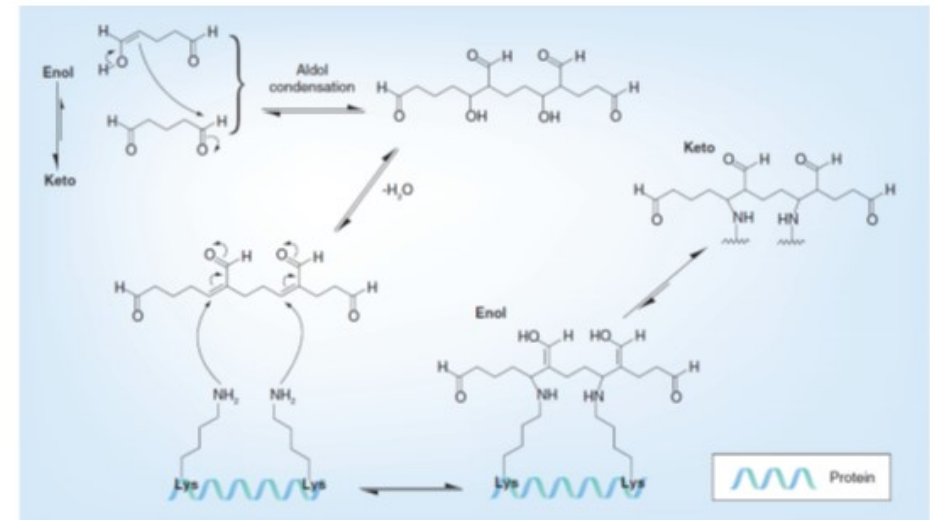
Inactivation by Glutaraldehyde

Glutaraldehyde is a saturated, five-carbon dialdehyde.

The carbonyl carbons are a nucleophile and attacks carbons in a nucleophilic addition reaction, just like formaldehyde.

Genomic DNA or RNA is the target of glutaraldehyde.

The exocyclic amino group on adenine (N6) is the reactive group, which attacks glutaraldehyde



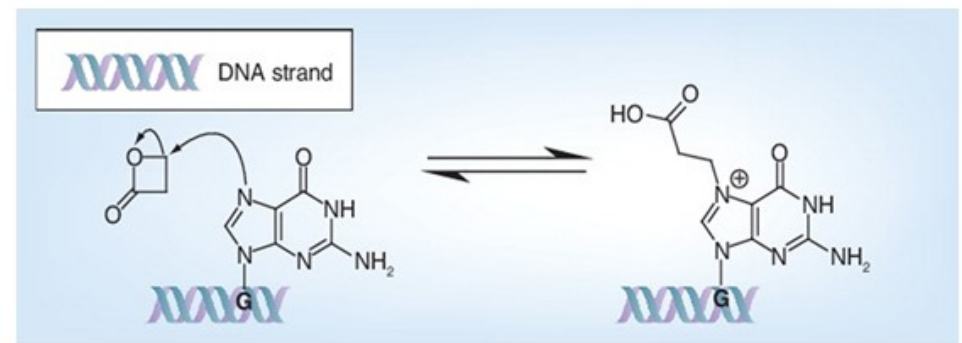
Reaction mechanism of glutaraldehyde with amino acids of proteins

Inactivation by β -propiolactone (BPL)

- Widely used to inactivate viruses for vaccine purposes
- Mainly acting as an alkylating agent on guanine of viral DNA or RNA

Mechanism of action of β -propiolactone with DNA or RNA (guanine).

The nucleophilic guanine of nucleic acids reacts with the electrophilic BPL through a nucleophilic substitution mechanism causing the ring opening of the strained BPL and the N-alkylation of guanine.



Reaction mechanism of BPL with DNA or RNA (Guanine)

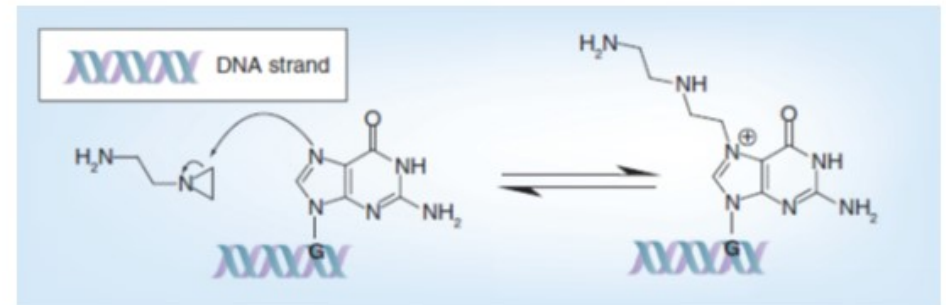
Inactivation Binary ethylene imine

Aminoethyl ethylene imine, also called BEI, which has two protonizable amino groups

BEI passes through the capsid and alkylates the viral genome

N7-guanine of the genome acts as a nucleophile and reacts with the electrophile BEI

The nucleophilic substitution reaction performed by N7-guanine causes an opening of the BEI ring and guanine becomes alkylated



Reaction mechanism of BEI with DNA or RNA (Guanine)

Denaturation by altering pH

Increasing or decreasing the pH causes protein denaturation



proteins adopt a different 3D structure




A low (acidic) or a high (alkaline) pH inactivate viruses via denaturation of the secondary structures of proteins


Alteration in conformation of viral proteins that are involved in attachment to and replication in a host cell

Contd...

pH also has an effect on the genomic level



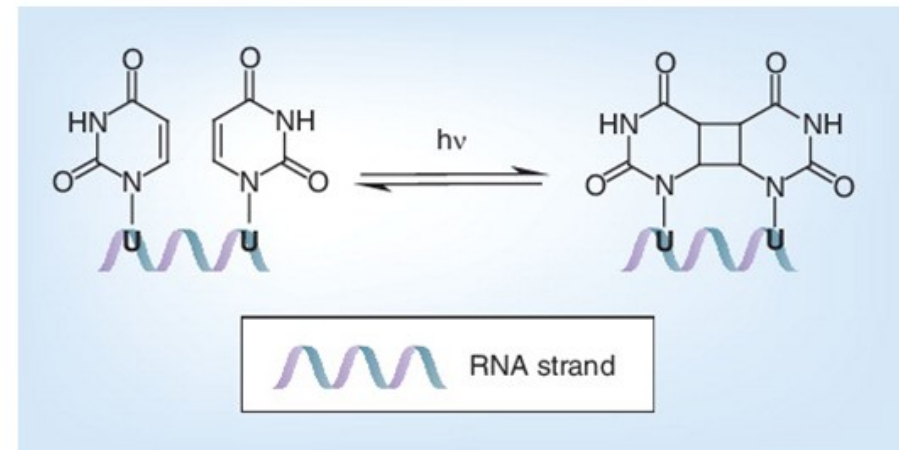
Hydroxyl group of RNA to the phosphorous center of each internucleotide linkage facilitates transesterification under strongly acidic or strongly basic conditions



Nucleophilic addition mechanism - oxygen attacks the adjacent phosphorus center, with a breakage of the phosphodiester bond as a consequence

Denaturation by Ultraviolet irradiation

- Extreme ultraviolet irradiation used for completely killing or inactivating turbid suspensions of bacteria and viruses in less than one second by exposing continuously.
- UV (200–280 nm) is absorbed by RNA and DNA bases and can lead to formation of dimers between two adjacent pyrimidines (uracil and thymine)
- Ultraviolet irradiation results in pyrimidine dimer formation between two adjacent or opposing pyrimidines.



Denaturation by Gamma irradiation

Direct result of a photon depositing energy into the target

- Direct result of a photon depositing energy into the target

Direct result of a photon depositing energy into the target

- Indirect damage via free radicals formed after breakage of covalent bonds.

Summary

Crosslinkers, such as formaldehyde and glutaraldehyde, or denaturing processes, such as pH and heat influence viral proteins and destroy the virion structure.

Killed vaccines based on these inactivation methods might not induce a proper immune response.

Alkylating agents, such as β -propiolactone (BPL) and binary ethylene imine (BEI), ultraviolet and gamma irradiation mainly influence the viral genome, preserving the virion structure.

These methods are suitable for generation of an efficient and safe KV vaccine.



Thanks

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