RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

Chemicals Changing the Specificity of Hydrogen Bonding:

There are many chemicals that after incorporation into DNA change the specificity of hydrogen -bonding. Those which are used as mutagens are nitrous oxide (HNO_2), hydroxylamine (HA) and ethyl-methane-sulphonate (EMS).

(a) Nitrous Oxide (HNO_2):

Nitrous oxide converts the amino group of bases into keto group through oxidative deamination. The order of frequency of deamination (removal of amino group) is adenine > cytosine > guanine. Cytosine deaminated to Uracil, which hydrogen bond to adenine

(i) Deamination of Adenine:

Deamination of adenine results in formation of hypoxanthine, the pairing behaviour of which is like guanine. Hence, it pairs with cytosine instead of thymine replacing AT pairing by GC pairing.

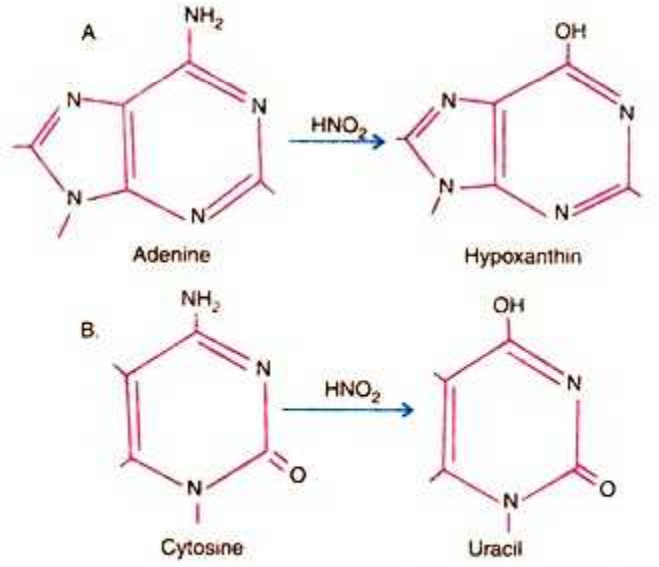


Fig. 9.8 : Deamination by nitrous oxide of adenine into hypoxanthin (A), and cytosine into uracil (B).

(ii) Deamination of Cytosine:

Deamination of cytosine results in formation of uracil by replacing – NH_2 group with -OH group. The affinity for hydrogen bonding of uracil is like thymine; therefore, C-G pair-ing is replaced by U-A pairing.

(iii) Deamination of Guanine:

Deamination of guanine results in formation of xanthine, the later is not mutagenic. Xanthine behaves like guanine because there is no change in pairing behaviour. Xanthine pairs with cytosine. Therefore, G-C pairing is replaced by X-C pairing.

(e) Hydroxylamine (NH_2OH):

Hydroxylamine (**NH2OH**) is a strong mutagen which modifies cytosine and adenine to N4-hydroxycytosine and N6-hydroxyadenine, respectively. Once these analogues are present in DNA or RNA, they may cause **transition** point **mutations**. Hydroxylamine is a mutagen that adds a hydroxyl group to C_4 nitrogen of cytosine and converts into a modified base via deamination (conversion from amine to keto form) which causes base pairs like thymine. The **deamination** of cytosine yields uracil. Unrepaired uracil residues will pair with adenine in replication, resulting in the conversion of a G–C pair into an A–T pair (a **GC → AT transition**). Therefore, GC pairs are changed into AT pairs.

iii. Alkylating Agents:

Addition of an alkyl group to the hydrogen bonding oxygen of guanine (N_7 position) and adenine (at N_3 position) residues of DNA is done by alkylating agents. As a result of alkylation, possibility of ionization is increased with the introduction of pairing errors. Hydrolysis of linkage of base-sugar occurs resulting in gap in one chain. This phenomenon of loss of alkylated base from the DNA molecule (by breakage of bond joining the nitrogen of purine and deoxyribose) is called depurination. Depurination is not always mutagenic. The gap created by loss of a purine can effectively be repaired

Following are some of the important widely used alkylating agents:

(a) Dimethyl sulphate (DMS)

(b) Ethyl methane sulphonate (EMS) -$CH_3CH_2SO_3CH_3$

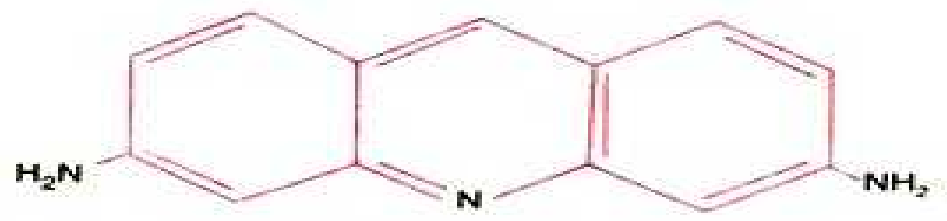
(c) Ethyl ethane sulphonate (EES) -$CH_3CH_2SO_3CH_2CH_3$

EMS has the specifity to remove guanine and cytosine from the chain and results in gap formation. Any base (A,T,G,C) may be inserted in the gap. During replication, chain without gap will result in normal DNA. In the second round of replication gap is filled by suitable base.

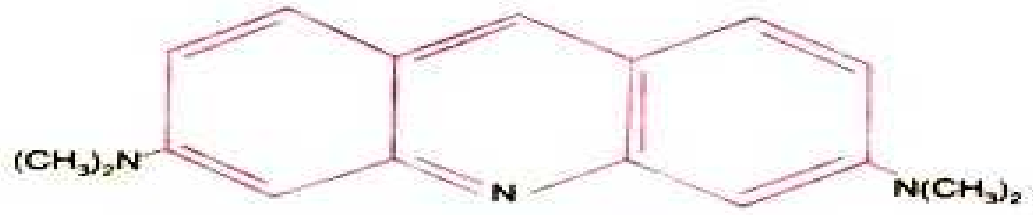
If the correct base is inserted, normal DNA sequence will be produced. Insertion of incorrect bases results in transversion or transition mutation. Another example is methyl nitrosoguanidine that adds methyl group to guanine causing it to mispair with thyamine. After subsequent replication, GC is converted into AT transition.

iv. Intercalating Agents:

There are certain dyes such as acridine orange, proflavine and acriflavin which are three ringed molecules of similar dimensions as those of purine pyrimidine pairs (Fig. 9.9). In aqueous solution these dyes can insert themselves in DNA (i.e. intercalate the DNA) between the bases in adjacent pairs by a process called intercalation.



Proflavin



Acridine Orange

Fig. 9.9 : Chemical structure of two mutagenic acridine derivatives.

Therefore, the dyes are called intercalating agents. The acridines are planer (flat) molecules which can be intercalated between the base pairs of DNA; distort the DNA and results deletion or insertion after replication of DNA molecule. Due to deletion or insertion of intercalating agents, there occur frameshift mutations