DNA Damage: Alkylation

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Alkylation is the reaction of electrophilic chemical compounds or alkylating agents with the nucleophilic centers in organic macromolecules. Alkylating agents include a large variety of chemicals, some of which are potent mutagens or carcinogens.

It is well known that DNA is a key structure which determines development and reproduction of the leaving organisms ranging from simple cells to humans. The sequence and structure of individual nucleotides in DNA are essential for the high degree of fidelity required for generating a “blueprint” during the process of storage and passing of genetic information from one organism to another. However, DNA is subject to alteration in structure and/or sequence of individual bases. These changes can result in errors during the steps of the DNA replication, recombination and repair, thus leading to the modification of the molecular structure of genetic material. Most of these modifications can be successfully removed by the DNA repair machinery. However, a significant amount of DNA remains unrepaired, which can lead to mutagenesis. The common source of the modifications in DNA, particularly the alteration in the nucleotide structure, can be found in the instability of specific chemical bonds of nucleotides under different physiological conditions, such as temperature or pH. Moreover, the DNA structure readily reacts with multiple chemical compounds and physical agents found in the environment. These mutagens and carcinogens can be produced by a variety of

chemical reactions, metabolism of other living forms, and also can be man-made. Modifications of the DNA molecular structure can be characterized as DNA damage. DNA damage is unavoidable and can be divided into two categories: 1) spontaneous, such as formation of mismatches, deamination of bases, loss of base, oxidative damage, and 2) environmental, such as ionizing radiation, UV radiation and chemical agents, including ubiquitous alkylating agents. DNA Alkylation, which is a part of the DNA damage, is the reaction of the alkylating agents with the nucleophilic centers, such as oxygen or nitrogen in the DNA base or backbone. The result of the reaction is the formation of the modified DNA bases called adducts or formation of the phosphotriesters in the case of the interaction of the agent with the oxygen in the DNA phosphodiester backbone. The adduct together with the opposite base is usually called a lesion.

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I. Alkylating agents and mechanism of the reaction with DNA

The fist chemical evidence of alkylation damage to DNA was reported in 1962 by the observation of 7-methylguanine as an *in vivo* product of dimethylnitrosamine administered to rats. Since that time, the list of alkylation agents and sites of alkylation has significantly increased, so that now we know that almost all nitrogens and oxygens of nucleotides in DNA can be modified. Alkylating agents include a large number of molecules, which can efficiently react with DNA causing structural modification of the base and phosphate group in DNA. The most common agents are alkyl sulfates, alkyl sulfonates, alkyl halides, dialkyl nitrosamine, alkyl nitrosoureas, acyl nitrosamides, mustards, diazo compounds, lactones, epoxides, etc (Figure 1). These agents can be divided into two major groups: monofunctional and bifunctional. The monofunctional

agents have only one reactive group which is involved in covalent interaction with the single center on DNA. The bifunctional agents have two reactive groups and have an ability to react with two centers on DNA. If the two centers are on the opposite strands of the DNA the reaction of a bifunctional agent, such as sulfur or nitrogen mustards, can produce an intrastrand cross-link. Many of the alkylating agent have a mutagenic effect and are known or suspected carcinogens. The reaction specificity of these compounds is different with different bases. Diazoalkanes react readily with guanosine and thymidine, but only under extreme conditions with adenosine and cytosine. In contrast alkyl sulfates, alkyl sulfonates, mustards, epoxides and nitroso compounds alkylate bases in the following order: G > A > C >> T. The rate of ethylation is much slower than that of methylation for all classes of alkylating agents except alkyl iodides, which readily alkylate G, A and C.

The main chemical mechanism of DNA alkylation is a S_N2 and S_N1 type reaction in which the electrophile (alkylating agent), reacts with the electron rich regions of the base residues and phosphodiester backbone. Figure 2 shows possible sites of alkylation on the DNA bases and the relative reactivity of these sites as measured by the presence of the alkylated base after the reaction with carcinogenic alkylating agents. The ring nitrogens, being more nucleophilic than the oxygens, are known to be more reactive with the alkylating agents. Thus, the N7 of guanine and N3 of adenine are being the most reactive. The DNA phosphodiester backbone can also undergo an alkylation reaction through interaction of the agent with the oxygen, which results in formation of phosphotriesters (Figure 2). However, not all alkylating agents react directly with the

DNA bases. Some of them, such as dialkylnitrosamines or vinyl chloride (group of R-halides), have to be metabolically activated.

II. Effect of DNA conformation on the alkylation

There are at least two significant differences between alkylation of nucleosides and polynucleotides. One is the presence of the phosphate in the phosphodiester bonds, which can form phosphotriesters, and the other is the effect of secondary structure on the availability of reactive sites. In general, the major sites of alkylation of single-stranded synthetic polynucleotides are the same as for nucleosides, but the extent of reaction is different. The neighboring base or the overall charge of the nucleotide can effect the nucleophilic potential of the DNA base. For example, if the guanine residue is flanked by an other guanine the negative electrostatic potential of the N7 position of guanine is enhanced, thus providing a better environment for the electrophilic attack of the alkylating agent. However, the increase of the negative charge of the alkylating moiety diminishes the nucleophilic potential of the base. Steric effects also play an important role in the reaction between the alkylating agents and the nucleophilic sites in DNA. For the normal right-handed (B-form) helix conformation the access to sites in DNA differs between the major and minor groove. For the guanine residue in the B-form DNA the O6 and N7 atoms are in the major groove, which can be easily accessed by the alkylating agent. In contrast, the relatively reactive N3 of the adenine lies in the minor groove, which is less accessible due to the steric implications of the B-form DNA.

III. Methods of identification of alkyl derivatives of nucleic acids

The initial identification and characterization of the alkylation products can be made using the UV absorption spectroscopy. The nature of the alkyl group introduced to the base does not affect the λ_{max} or λ_{min} significantly. However, the position of the base modification results in the spectral changes that makes it possible to distinguish among derivatives. In addition to spectra maxima and minima of the cationic and anionic forms, the shape of the spectra changes significantly depending on the base modification. The characteristic shoulders and alteration in the curve profile can be assigned to a particular adduct. Additional identification can be performed using other methods such as mass spectrometry, nucleic magnetic resonance and infrared spectroscopy. The detailed description of these methods and their use for identification of alkyl derivatives can be found in further reading and references within.

IV. Cytotoxic and mutagenic effects of alkylation. Methylation.

Mutation can be described as a change of one base to another, leading to a change in coding information. The change in the base structure which lethal to the cell, can be characterized as a cytotoxic.

Direct alkylation of DNA, particularly methylation, acting through the covalent modification of the base, has the ability to generate miscoding base derivatives and lesions that block replication. The major adducts generated in double-stranded DNA by the methylating agents such as methyl methanesulphonate (MMS), dimethylsulphate (DMS) and methyl iodide (MeI) are: 7-methylguanine (7-meG), 3-methyladenine (3-meA), 3-methylguanine (3-meG) and O^6-methylguanine (O^6-meG) (Figure 3A). In single

stranded DNA, these methylating agents form 1-methyladenine (1-meA) and 3-methylcytosine (3-meC) (Figure 3A). The formation of these adducts in the single stranded, but not in double-stranded DNA is due to the fact that these modification sites are involved in base-pairing and are therefore protected from alkylation. 3-meA, 3-meG and 3-meC have the ability to block DNA replication, thus having cytotoxic effects. In contrast, O^6-meG and O^4-methylthymine (O^4-meC) are miscoding base derivatives which mispair during replication thus leading to a possible mutations. Environmental mutagens such as 1,2-dimethylhydrazine, diazoquinones and tert-butylhydroperoxide generate methyl radicals which readily react with the guanine residue to form 8-methylguanine, which also has a high miscoding potential. However, the most common methylation product in DNA, 7-meG, is not mutagenic and basepairs normally in polynucleotides. Most of the adducts produced by methylation can be efficiently removed by a variety of DNA glycosylases through the base excision repair mechanism (BER). This process involves cleavage of base-sugar bond in order to release the modified base and generate apurininc/apyrimidinic site which is repaired by endonucleases. This is followed by the action of the DNA polymerase which incorporates the correct base. In contrast, O6-methylguanine is corrected by the direct transfer of the methyl group to a cysteine residue of the methyltransferase repair enzyme. Intrastrand cross-links (Figure 3B), generated by the bifunctional alkylating agents, such as nitrogen mustard, represents an important class of DNA damage, since it prevents DNA strand separation, which is crucial to the processes of replication and transcription. The cell has the ability to repair this damage through the mechanism of nucleotide excision repair (NER), which uses multiple enzymes.

The nitroso compounds and hydrocarbons, which are well known environmental carcinogens, react through a number of metabolic intermediates. However, the most effective direct alkylating agents, such as alkyl sulfates (Figure 1), in turn, are poor carcinogens. In contrast, the metabolically activated alkylating agents are very efficient carcinogens. One of the widely used common chemical, which is carcinogenic in man and experimental animals, is vinyl chloride (Figure 3C). The industrial use of vinyl chloride is estimated to be about 4×10^9 kg/year in the United States. The exposure to vinyl chloride is generally by inhalation and results in an angiosarcoma of liver or other tumors in the brain, lung and hematolymphopoietic system in humans. Vinyl chloride is readily metabolized into chloroethylene (CEO) oxide which rapidly changes to chloroacetaldehyde (CAA) (Figure 3C). Both CEO and CAA are highly mutagenic and carcinogenic and react with the DNA bases to form etheno derivitives (e.g $1,N^6$-ethenoadenine and $3,N^4$-ethanocytosine(Figure 3A)). These adducts have a high miscoding potential and also an ability to block DNA replication.

Glossary: DNA – Deoxyribonucleic acid; nucleoside – consists of a purine or pyrimidine base linked to a pentose; nucleotide – consists of a nitrogeneous base, a sugar, and one ore more phosphate groups; adduct – structurally modified DNA nucleotide; alkylating agent – chemical compound which has ability to react with the DNA through the reaction of alkylation;

Further Reading:

Singer, B. (1975) The Chemical Effects of Nucleic Acid Alkylation and Their Relation to Mutagenesis and Carcinogenesis. Prog. Nucleic Acid Res. Mol. Biol. 15: 219-284

Singer, B. and Grunberger, D. (1983) Molecular Biology of Mutagens and Carcinogens. Plenum Press, New York, NY

Lindahl, T. and Sedgwick, B. (1988) Regulation and Expression of the Adaptive Response to Alkylating Agents, Ann. Rev. Biochem. 1988 57:133-57

Sedgwick, B. and Lindahl, T. (2002) Recent Progress on the Ada Response for Inducible Repair of DNA Alkylation Damage. Oncogene, 21: 8886-8894

Biographical Statement

Dr. B. Singer is a Research Professor Emeritus at the Virus Laboratory and Department of Molecular Biology at the University of California, Berkeley and presently Senior Scientist at Lawrence Berkeley National Laboratory. She has co-authored the text, "Molecular Biology of Mutagens and Carcinogens", as well as numerous papers on chemistry, molecular biology and virology. In 1957, together with her husband professor H. Fraenkel-Conrat, they discovered that all the genetic information for tobacco mosaic virus was coded by RNA alone. Her current research interests are on the chemical reactions of environmental carcinogens. Dr. Anton B. Guliaev is a Physicist Scientist at Lawrence Berkeley National Laboratory, Berkeley, CA.

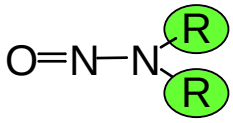
Legends for Figures

Figure 1. Structural formulas of alkylating agents. The alkyl groups are indicated by R. Circled R shows which group of the molecule is being transferred. The opening of the ring for the cyclic compounds is indicated by the line and the whole molecule act as a substituent.

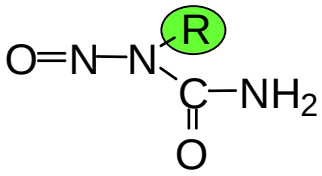
Figure 2. Structural formulas of the DNA bases with the possible sites of the modification by alkylating agents indicated by red arrows. dR indicates sugar. The table on the right shows the percent of total alkylation after reaction with the carcinogenic alkylating agent.

Figure 3. A) Selective structures of the DNA adducts formed by the alkylation reaction. **B)** Intrastrand cross-link product of the reaction of nitrogen mustard with N-7 of guanine. **C)** Mechanism of the metabolism of vinyl chloride

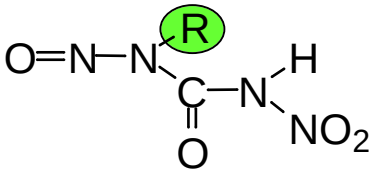
N-Nitroso compounds and diazoalkanes



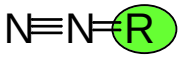
Dialkyl Nitrosamines (R = CH_3-, CH_3CH_2-)



N-Alkyl-N-nitrosureas (R = CH_3-, CH_3CH_2-)

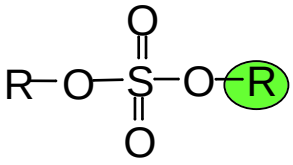


N-Alkyl-N'-Nitro-N-Nitrosoguanidine (R = CH_3-)

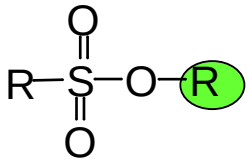


Diazoalkanes(R = CH_3-)

Alkyl sulfates and Halides



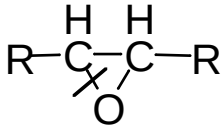
Dialkyl Sulfates (R = CH_3-, CH_3CH_2-)



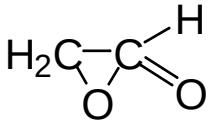
Alkyl Alkane Sulfonates (R = CH_3-, CH_3CH_2-)

Alkyl Halides (R = CH_3-, CH_2CH-)

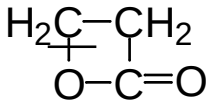
Cyclic Compounds



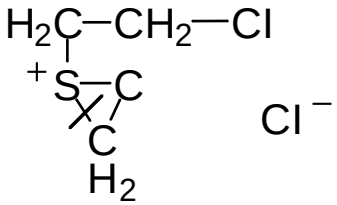
Epoxides (R = CH_2CH-, CH_3CHCH-)



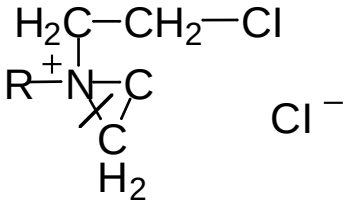
Glycidaldehyde



Lactones (β-propiolactone)

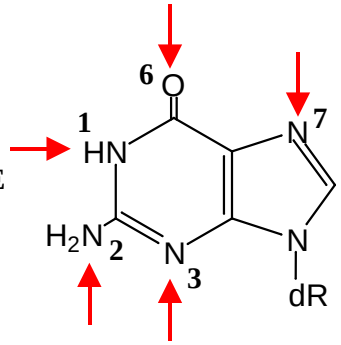


S-mustards (mustard gas)

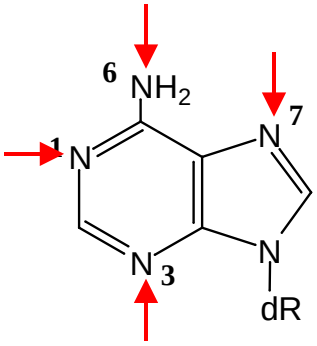


N-mustards (R = NH_2)

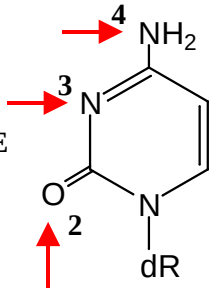
GUANINE



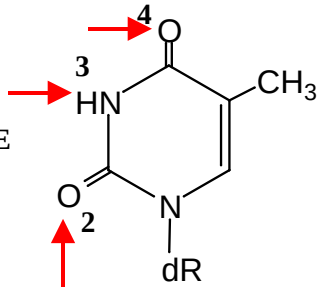
ADENINE



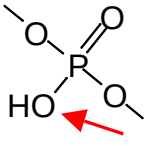
CYTOSINE



THYMINE



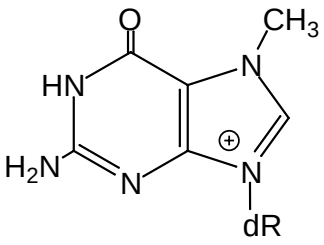
PHOSPHATE



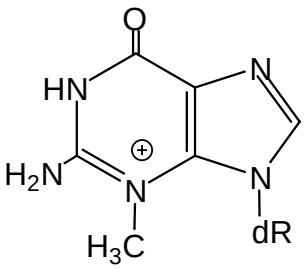
Carcinogenic alkylating agent / Adduct	% of total alkylation: DMN MNU SDMH	DEN ENU	MMS
N^3-Alkylguanine	**0.6**	**1.5**	**0.7**
O^6-Alkylguanine	**3-6**	**8**	**0.3**
N^7-Alkylguanine	**69**	**12**	**8.3**
N^1-Alkyladenine	**0.8**	**0.1**	**1.2**
N^3-Alkyladenine	**4**	**4**	**11**
N^7-Alkyladenine	**1.5**	**0.6**	**1.9**
O^2-Alkylcytosine	**0.1**	**2**	
N^3-Alkylcytosine	**0.5**	**0.3**	
O^2-Alkylthymine	**0.1**	**7**	
N^3-Alkylthymine	**0.3**	**0.4**	
O^4-Alkylthymine	**0.1**	**2.5**	
Triester	**12**	**58**	**1**

DMN - Dimethylnitrosamine
MNU - Methylnitrosourea
SDMN - 1,2-Dimethylhydrazine
DEN - Diethylnitrosamine
ENU - Ethylnitrosourea
MMS - Methyl methanesulphonate

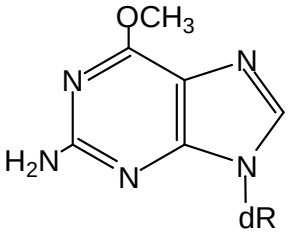
A)



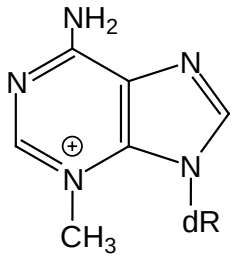
7- methylguanine



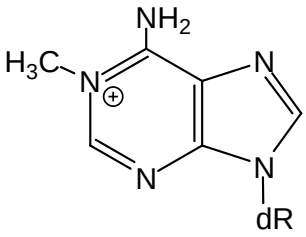
3- methylguanine



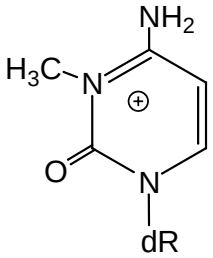
O^6- methylguanine



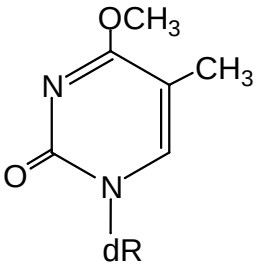
3- methyladenine



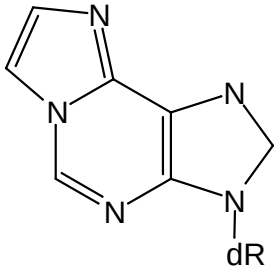
1- methyladenine



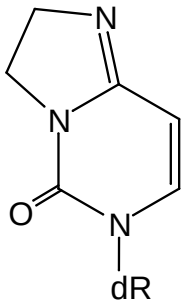
3- methylcytosine



O^4- methylthymine

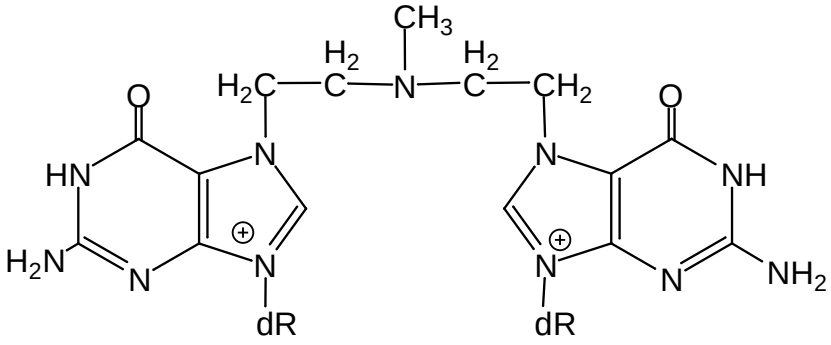


$1,N^6$- ethenoadenine

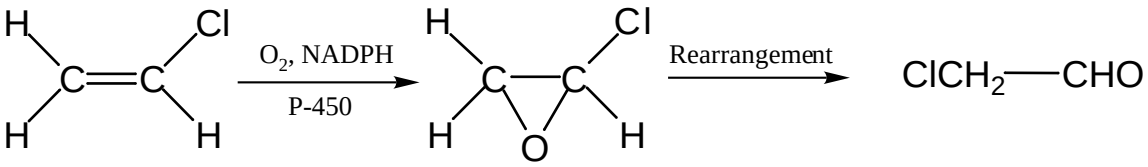


$3,N^4$- ethanocytosine

B)



C)



vinyl chloride

chloroethylene oxide

chloroacetaldehyde