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Normal and Abnormal Photobiologic Responses

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3 MOLECULAR CHANGES IN THE NUCLEIC ACIDS PRODUCED BY ULTRAVIOLET AND VISIBLE RADIATION

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ABSTRACT

Radiation can produce two general types of damage in the nucleic acids: base damage and chain breaks.

Base damage to the pyrimidines (and purines) can be of two major types: unimolecular and bimolecular. Examples of unimolecular reactions are the addition of a hydrogen and a hydroxyl group to the 5–6 double bond of the pyrimidines (photohydrate), the reduction of the 5–6 double bond of thymine, and the oxidation of the methyl group of thymine. The number of possible different bimolecular reactions is almost unlimited. The better-known reactions involve the covalent linkage of two pyrimidines by one bond (an adduct) or by two chemical bonds (a dimer) and the photochemical addition of other compounds to the pyrimidines such as amino acids (cross-linking of proteins and nucleic acids), and drugs such as psoralen (photosensitized reactions).

DNA chain breaks (produced primarily by certain types of photosensitized reactions and by x-rays) are also of two major chemical types, resulting from: (1) the cleavage of a phosphate ester bond, and (2) the cleavage of the carbon atom skeleton of a deoxyribose group. There are several different chemical types of each of these two classes of DNA chain breaks.

The significance of emphasizing the multiplicity of the nature of the damage to DNA produced by ultraviolet and sensitized visible radiation is that the number and types of enzymes required to repair this radiation damage will, of course, depend upon the chemical nature and complexity of the damage that is produced.

I. INTRODUCTION

Although radiation produces chemical alterations in all types of biologic molecules, the central role that deoxyribonucleic acid (DNA) plays in the molecular biology of a cell has implicated DNA as the most important target for radiation, and whose alteration leads to the production of mutations and/or to cell death.[1,2] For this reason, this paper will be limited to a discussion of the photochemical alterations of DNA.

DNA is composed of two intertwined polymeric chains of alterating deoxyribose and phosphoric acid residues to which are attached four types of heterocyclic

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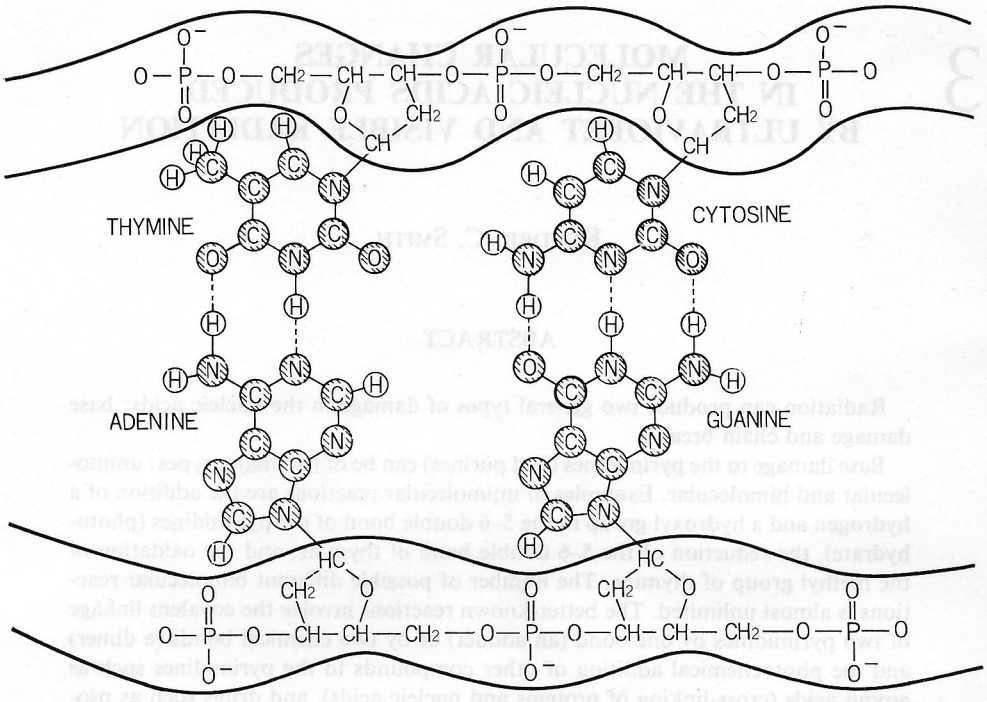

Fig. 1. Atomic structure of DNA.

organic bases: two different purines (guanine and adenine) and two different pyrimidines (thymine and cytosine) (Fig. 1). As may be predicted from the gross morphology of double-stranded DNA, radiation produces two general types of damage in DNA: chemical alteration of the heterocyclic organic bases and scissions in the sugar–phosphate backbone of DNA.

II. BASE DAMAGE

Base damage to the pyrimidines and purines can be of two major types: unimolecular and bimolecular.

A. Unimolecular Reactions

Since the pyrimidines are about 10-times more sensitive than are the purines to photochemical alteration by ultraviolet (UV) radiation, the present discussion will be largely restricted to the photochemical reactions of the pyrimidines.

If the pyrimidines are irradiated *in vitro* with large doses of UV radiation at 254 nm, a series of chemical changes take place which ultimately yield carbon dioxide, water, and ammonia. These cataclysmic chemical events are of little biologic importance since they require supralethal doses of radiation. Rather, the photochemical reactions that are of biologic significance are the primary and possibly the

secondary chemical changes in the nucleic acid bases, since these changes can be produced by biologically relevant doses of UV and sensitized visible radiation. A number of the primary photochemical reactions of the pyrimidines are now known (for recent reviews see Refs. 3–6).

1. *Hydration.* The first photochemical reaction to be discovered that did not cause the destruction of the pyrimidine ring was the addition of a hydrogen atom and a hydroxyl group (i.e., the component parts of a molecule of water) to the 5–6 double bond of the pyrimidines, uracil and cytosine, to form a product referred to as a photohydrate (Fig. 2). The saturation of the 5–6 double bond of the pyrimidines

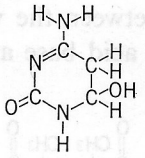

Fig. 2. Photohydrate of cytosine.

destroys its characteristic UV absorption spectrum and, in the case of cytosine, weakens the bonding of the amino group so that deamination or amino group-substitution[7] reactions can readily occur. The hydrated pyrimidines can, by the elimination of a molecule of water, be converted back to the original product with good efficiency by altering the pH or by raising the temperature of the solution. The photohydrate of thymine is particularly unstable. There have been a few reports that certain UV-inactivated biologic systems show a small amount of reactivation by mild heating.[8]

2. *Reduction.* The addition of two atoms of hydrogen to the 5–6 double bond of thymine in DNA has been demonstrated *in vitro*[9] (Fig. 3). The biologic importance of this reaction is not known.

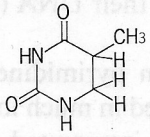

Fig. 3. Dihydrothymine.

3. *Oxidation.* When thymine was UV irradiated in solution, 5-formyl uracil was isolated[10] (Fig. 4). Aldehyde groups are well known for their reactivity with amino groups. If 5-formyl uracil is formed by UV irradiation of DNA *in vivo*, it has the interesting possibility of reacting with amino groups to produce a covalent cross-link between DNA and protein (see below).

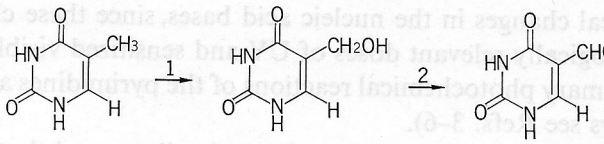

Fig. 4. Formation of 5-hydroxymethyl uracil and 5-formyl uracil from thymine.

B. Bimolecular Reactions

While the number of possible unimolecular photochemical reactions of the purines and pyrimidines is limited, the number of possible bimolecular reactions is almost unlimited. For convenience we may divide the bimolecular reactions into two classes: (1) those occurring between the various nucleic acid bases, and (2) those occurring between a nucleic acid base and some other type of compound.

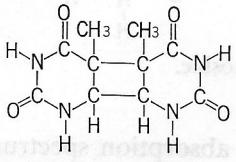

Fig. 5. Cyclobutane-type thymine dimer.

(1) The best known bimolecular reaction is the linkage of two pyrimidine residues by two covalent bonds to form a cyclobutane-type dimer (Fig. 5), so-called because of the four-membered ring that links the two pyrimidine rings. Such dimers are formed *in vivo* by sublethal doses of UV radiation between adjacent thymine residues, between adjacent cytosine residues, and mixed dimers are formed between adjacent cytosine and thymine residues. Because of the unique chemical stability of the cyclobutane-type dimer of thymine, and hence the ease with which it can be isolated and assayed, it is the DNA photoproduct that has been most extensively studied both from the point of its chemistry and of its biologic importance. In fact, the latter studies led to the discovery of one of the mechanisms by which cells are able to repair radiation damage to their DNA (see Chapter, *The Cellular Repair of Radiation Damage*).

Other types of adducts between pyrimidines are also known (Figs. 6, 7). In general, these products are produced in much lower yield than are the cyclobutane-type dimers but this should not be interpreted, *a priori*, to mean that they may be of less biologic importance. It is well known that the yield of a given DNA photoproduct is markedly affected by the experimental conditions (i.e., the physical state of the DNA).[3,11] For example, in UV-irradiated vegetative bacteria the cyclobutane-type thymine dimer (Fig. 5) is the major photoproduct produced, however, in UV-irradiated spores of the same strain of bacteria (*Bacillus subtilis*) very little cyclobutane-type dimer is formed; rather, a new type of adduct between two thymine residues is formed (so-called "spore photoproduct"; Fig. 7). The DNA is

known to be in a different physical state in the vegetative and spore forms of bacteria.[3]

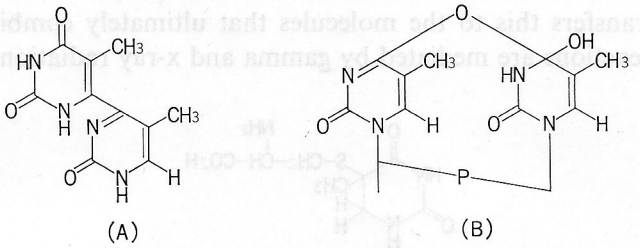

Fig. 6. Tentative structures of some noncyclobutane-type photoproducts of thymine. (A) 6,4′-'5′-methylpyrimidin-2′-one'—thymine; a minor product formed when thymine is irradiated in frozen solution and subsequently heated in acid. (B) TpT[4], a minor product formed when thymidylyl-thymidine (TpT) is irradiated in solution.

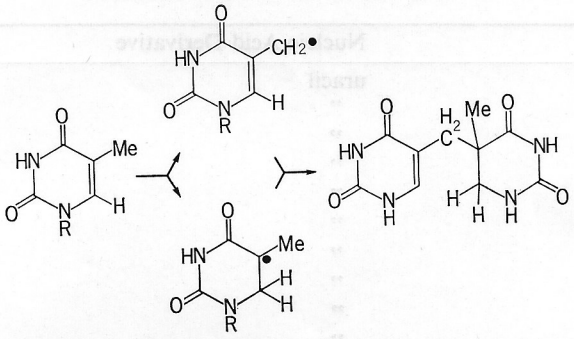

Fig. 7. The formation of 5-thyminyl-5,6-dihydrothymine by the addition of two different radicals of thymine. A major photoproduct produced in UV-irradiated bacterial spores.

(2) Although the possible number of different types of bimolecular reactions between the different pyrimidines and/or purines is limited, the number of possible reactions between the nucleic acid bases and other types of compounds is almost unlimited. The first such type of heteroadduct reaction to be discovered *in vivo* was the cross-linking of DNA and protein.[12] It was produced both by exposure to short wavelength UV radiation (254 nm) and to visible light in the presence of a dye such as acridine orange. The biologic importance of this type of reaction has been well documented.[13,14] A photochemical reaction between thymine and the amino acid cysteine has been observed that may be one mechanism for the covalent linking of DNA and protein *in vivo* (Fig. 8).

Eleven of the common amino acids have been shown to combine photochemically with the pyrimidine base uracil, but the chemical structures of most of these adducts are not yet known. The Table lists a number of compounds that have been

shown to combine with the nucleic bases. It should be noted that certain of these reactions are produced by the direct absorption of UV radiation or of near-UV and/or visible radiation, while other reactions require the intervention of a third molecule which acts as a sensitizer for the reaction (i.e., it absorbs the radiation energy and transfers this to the molecules that ultimately combine). Still other bimolecular reactions are mediated by gamma and x-ray radiation.

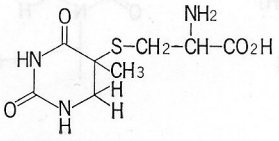

Fig. 8. 5-S-cysteine, 6-hydrothymine.

Table. Radiation-induced formation of adducts to nucleic acid derivatives.

A. SHORTWAVE ULTRAVIOLET RADIATION

Compound	Nucleic Acid Derivative	Reference
glycine	uracil	28
serine	"	28
phenylalanine	"	28
tyrosine	"	28
tryptophan	"	28
cystine	"	28
methionine	"	28
histidine	"	28
arginine	"	28
lysine	"	28
cysteine	uracil	18, 28, 30
"	TpBrU	16
"	thymine	29
"	polyribonucleotides	32
"	polydeoxyribonucleotides	32
"	RNA	32
"	DNA	32
cysteamine	iodouracil	26
tyrosine	DNA, RNA	32
serine	"	32
threonine	"	32
methionine	RNA	32
alanine	caffeine	12
serine	"	12
threonine	"	12
leucine	"	12
norleucine	"	12
norvaline	"	12
alanylglycine	"	12

Compound	Nucleic Acid Derivative	Reference
amines	caffeine	13, 33
,,	purine	42
,,	dimethyluracil	15
poly-L-lysine	,,	15
alcohols	purine, purine nucleosides	11, 35
,,	DNA	2, 34
,,	pyrimidines	23, 37
water	uracil	27
HCN	uracil, RNA	14
ethylene, propylene	uracil, uridine	21

B. LONGWAVE ULTRAVIOLET RADIATION

Compound	Nucleic Acid Derivative	Reference
benzo[a]pyrene	DNA	36
,,	thymine	3
,,	1-methylcytosine	7
acetophenone	purines, pyrimidines	10
benzophenone	,,	10
furocoumarins	DNA, RNA	22
,,	pyrimidines	24
acrylonitrile	,,	17

C. SENSITIZED ADDITION REACTIONS

Compound	Nucleic Acid Derivative	Sensitizer	Reference
alcohols	purines	acetone	35
,,	DNA	,,	2, 34
cysteine	uracil	benzophenone	18
,,	,,	acetone	18
,,	,,	riboflavin	18

D. GAMMA AND X-RAYS

Compound	Nucleic Acid Derivative	Reference
amines	caffeine	13, 33
fluorescein	DNA	1
phenylalanine	RNA	40
,,	DNA	6
leucine	,,	6
tryptophan	RNA, DNA	41
3,4-benzopyrene	DNA	8, 25
protein	phage T1 DNA	4
alcohols	purines, purine nucleosides	35
,,	thymine	5, 43
water	uracil	31
N-ethylmaleimide (NEM)	DNA	19
,,	thymine	38
formate	cytosine	20
nitrofurans	DNA	9
triacetoneamine-N-oxyl (TAN)	,,	39

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The biologic importance and numerical magnitude of this type of heteroadduct reaction between nucleic acid bases and other classes of compounds is just beginning to be properly appreciated. Since DNA does not exist in pure form within a cell but is in intimate contact with proteins, lipids, carbohydrates, and small

molecular precursors and metabolites, it seems most probable that more and more examples of this type of reaction will be found to play a major role in the photobiology and radiation biology of cells under many different experimental conditions.

III. DNA CHAIN BREAKS

Primary chain breaks are produced in low yield by UV radiation but are produced in high yield by ionizing radiation (i.e., x-rays) and by certain photochemical reactions mediated by near-UV radiation that involve sensitizers (e.g., benzophenone[15]). There has been a tendency by many to think that there is just one type of DNA chain break. Rather, there are many ways to break the DNA chain, leading to quite different chemical groups at the two ends of the break.[16,17] From Figure 9 it can be seen that there are several places where the phosphoric acid ester groups

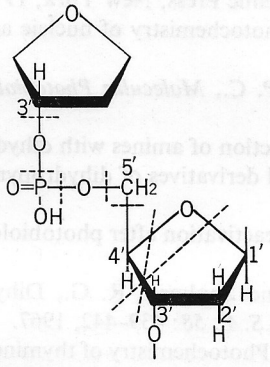

Fig. 9. Possible mechanisms for the formation of chain breaks in DNA.

can be cleaved and several ways in which the deoxyribose can be disrupted. Consistent with the observation that there are several ways in which a DNA chain can be broken, several distinct mechanisms are now known for the repair of radiation-induced DNA chain breaks (see Chapter, *The Cellular Repair of Radiation Damage*).

IV. CONCLUSIONS

While it is easy to understand why chemists should be interested in the numerous ways in which the nucleic acids can be altered by radiation, it should be stressed that knowledge of these changes is of great importance to biologists in their quest to understand the biologic effects of radiation. The survival of cells exposed to radiation is critically dependent upon their ability to repair radiation damage to their DNA (see Chapter, *The Cellular Repair of Radiation Damage*). Since enzymes show a large degree of specificity for the substrates upon which they act, the chem-

ical nature and complexity of the damage produced in DNA by radiation will determine the biologic impact of such damage by determining the number and types of enzymes that will be required to repair the damage.

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