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BIOCHEMICAL EFFECTS OF ULTRAVIOLET LIGHT ON DNA†

KENDRIC C. SMITH

Department of Radiology, Stanford University School of Medicine, Palo Alto, California, U.S.A.

SOME of the biological effects of UV irradiation can now be explained in terms of specific chemical and physical changes in DNA. The purpose of this report is to summarize the different photochemical lesions produced in DNA and to assess where possible their biological importance.‡

Effects of UV on Deoxyribose

Carbohydrates show essentially no UV absorption at wavelengths above 2300 Å, and therefore would not be expected to undergo any significant photochemical reactions when irradiated with light of wavelengths greater than 2300 Å. This would appear to be the case, although the photochemistry of carbohydrates has not been systematically studied in more recent years. Earlier reports on the photochemical alteration of carbohydrates are questionable because of the failure adequately to filter out wavelengths of light below 2300 Å.⁽⁴⁾

Effects of UV on Purines

Purines are approximately tenfold more resistant to photochemical alteration than are the pyrimidines. Because of this difference in sensitivity to photochemical alteration, the implication has been that the photochemistry of the purines is not important biologically since presumably by the time a significant amount of purine damage has occurred, the cells would already have died from damage produced in the pyrimidines. Although statistically this hypothesis has much in its favor the biological importance of purine photochemistry should not be so quickly dismissed. Although the absorption of UV by the purines does not result in the photochemical alteration of the purine ring with a high efficiency, some of the absorbed energy may well be transferred to other systems and might account, for example, for the chain breakage observed in irradiated polynucleotides. The transfer of energy from adenine has been implicated in the formation of a thymine radical in irradiated poly dAT.⁽⁸⁾

Hydration Products of the Pyrimidines

When uracil and cytosine and their derivatives are irradiated in solution they lose their characteristic UV absorbancy but this can be largely regenerated by heat or acid treatment. It was postulated that the hydration of the 5-6 double bond of uracil could account both

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‡For a more comprehensive coverage of certain areas of the photochemistry of the nucleic acids the recent reviews by J. K. Setlow,⁽¹⁾ R. B. Setlow,⁽²⁾ Shugar,^(3,4) Smith^(5,6) and Wacker,⁽⁷⁾ should be consulted.

for the loss of the absorption spectrum and the reversibility by subsequent treatment with acid or heat.⁽⁹⁾ The ultimate proof of this postulate came when 6-hydroxy, 5-hydrouracil (Fig. 1) was synthesized and was shown to be identical with the reversible radiation product of uracil⁽¹⁰⁾ Direct chemical evidence for the photochemical hydration of cytosine derivatives

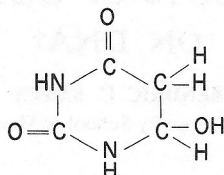


FIG. 1. 6-Hydroxy, 5-hydrouracil.

is now also available.^(11,12) There is no direct evidence, however, for the formation of hydrates of thymine.

The formation of the water addition photoproduct of cytosine in irradiated denatured DNA has been inferred from the appearance of a heat-reversible absorption peak around 2400 Å.⁽¹³⁾ Irradiated native DNA, however, showed no such heat-reversible absorption peak. These data suggest that hydrates of cytosine are probably not formed in irradiated double-stranded DNA but are formed in single-stranded DNA.

During replication and/or transcription of the DNA, there may be short regions of single-strandedness, and in these regions the formation of pyrimidine hydrates may well be of importance. The possible role of pyrimidine hydrates in causing mutations has been demonstrated *in vitro*. When poly-cytidylic acid was irradiated with UV, its coding properties in an RNA polymerase system were altered.⁽¹⁴⁾ The irradiated polymer lost its ability to code for the incorporation of guanylic acid but then coded for the incorporation of adenylic acid. The increase in adenylic acid incorporation was heat reversible under conditions known to repair pyrimidine hydrates and for this reason it was suggested that the code change might be the result of the formation of cytosine hydrates.⁽¹⁴⁾

Although present evidence indicates that cytosine hydrates are not formed in irradiated double-stranded DNA, their formation in single-stranded regions of the DNA may well be of significance in the production of mutations which may or may not be lethal.

Cyclobutane-type Dimers of Thymine, Cytosine and Uracil

The formation of the thymine dimer results in the loss of the characteristic UV absorption of thymine but contrary to the results for the pyrimidine hydrates the dimer is not reversed to monomeric thymine by treatment with heat. The dimer, however, can be converted back to monomeric thymine by irradiation with light of short wavelength.

To form the thymine dimer (Fig. 2), two thymine molecules are linked to each other by

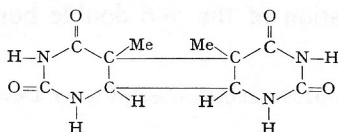


FIG. 2. Thymine dimer (cyclobutane-type).

carbon-carbon bonds between their respective five and six carbon atoms thus forming a cyclobutane ring between the two thymines.⁽¹⁵⁾ There are six possible isomers of the thymine dimer⁽¹⁶⁾ and five of these have now been isolated from irradiated thymine oligomers.^(17,18) Certain of these isomers are stable to acid hydrolysis while others are not.

There is a wavelength dependency for the formation and monomerization of the cyclobutane-type thymine dimer, such that after a sufficient dose of UV a photosteady state between monomer and dimer is reached that is characteristic for the wavelength used in the irradiation. At the longer wavelengths (around 2800 Å) the formation of the dimer is favored while at the shorter wavelengths (around 2400 Å) the monomer is favored. This response is due to differences in the absorption spectra of thymine and its dimer and in the quantum yields for the formation and splitting of the dimer.^(13,19,20)

Five other pyrimidine dimers are also known. These are the dimers of uracil, cytosine, uracil-thymine, cytosine-thymine, and uracil-cytosine (numerous references cited in No. 6). Because these dimers have the property of short wavelength reversal they are believed to have the same skeletal structure as the cyclobutane-type thymine dimer, but direct proof of this postulate is lacking.

As with the thymine dimer the short wavelengths are also more efficient in monomerizing the uracil dimers, however, the uracil reaction is complicated by the production of hydrates which interfere with dimer production. The isolation of cytosine dimers is complicated not only by the competition of the hydrate reaction but also by the fact that cytosine deaminates readily when its 5-6 double bond is saturated. Cytosine dimers are therefore readily converted to uracil dimers. Cytosine dimers are formed at lower rates than are thymine dimers but they are monomerized at more rapid rates by short wavelength radiation than are thymine or uracil dimers.⁽²¹⁾

Certain of the lines of evidence that indicate that the thymine dimer is of biological importance are the following:

1. The short wavelength reversibility of the thymine dimer has been used to show that much of the inactivation of transforming DNA that is brought about by irradiation at 2800 Å is reversed by a second irradiation at 2400 Å. In this manner, it has been determined that after high doses of UV at 2800 Å about 50% of the inactivation of *Hemophilus influenzae*-transforming DNA can be attributed to the production of thymine dimers.⁽²²⁾
2. Bacterial cells that have been irradiated with UV show an increased survival if they are additionally irradiated with visible light.⁽²³⁾ This process is known as photoreactivation. The enzyme responsible for this phenomenon has been isolated⁽²⁴⁾ and shown to be specific for the repair of cyclobutane-type pyrimidine dimers.^(25,26) If this enzyme has the same specificity *in vivo* then it argues for the biological importance of pyrimidine dimers. It should be emphasized, however, that photoreactivation does not cause the complete reversal of all UV damage to a cell, suggesting that there are biologically important photochemical lesions produced in DNA besides the cyclobutane-type pyrimidine dimers.
3. Certain strains of *E. coli* are very sensitive to killing by UV while others are very resistant. The observation has been made that the resistant strains have the ability to cut out thymine dimers from their DNA^(27,28) and to undergo repair replication of their DNA⁽²⁹⁾ whereas the sensitive strains are unable to perform this "cut and patch" type of repair. While the pyrimidine dimers may be of biological importance to an organism

that is incapable of repairing them, they are probably of little biological importance to those organisms that have efficient repair mechanisms.

Since much of this work was done prior to the general recognition of the involvement of cytosine in cyclobutane-type dimers some of the biological effects assigned to thymine dimers by the above experiments should probably be reassigned to dimers involving cytosine.

The majority of our knowledge on the photochemistry of the nucleic acids concerns the thymine dimer and the largest amount of evidence supporting the biological importance of a given photoproduct is also concerned with the thymine dimer. The sheer volume of these data has tended to imply that other types of photochemical lesions in DNA are not of biological importance. The thymine dimer is unquestionably of major biological importance under certain experimental conditions, but recent data indicate that it is not of major importance in all situations. The photochemical yield and relative biological importance of the thymine dimer is different for different cells and can even change for a given cell under different growth and irradiation conditions.⁽³⁰⁾ Therefore, it must be remembered that the absolute biological importance of any photoproduct depends upon (1) whether or not it is formed under a particular set of experimental conditions and (2) if formed, whether or not the particular system under study is capable of repairing the lesion.

Other Photochemical Reactions of the Pyrimidines

Many pyrimidine photoproducts other than the hydrates or the cyclobutane-type dimers are produced both *in vivo* and *in vitro*. The structure of most of these new photoproducts is unknown and their identity is based upon chromatographic properties, (numerous references cited in No. 6). The relative biological importance of these photoproducts remain to be determined.

Effect of UV on the Molecular Weight of DNA (Chain Breakage)

There was no dramatic effect of DNA base composition upon the efficiency of DNA chain breakage brought about by the UV irradiation of DNA *in vitro*.⁽³¹⁾ The dose of UV required to reduce the molecular weight of *D. pneumonia* DNA by 50% was about 100 times that required to reduce the transforming activity of the streptomycin marker in this DNA to the same extent.⁽³¹⁾ At the dose of UV required to kill 90% of a population of phage T7, no chain breaks were detected.⁽³²⁾ Therefore, current evidence suggests that at low doses of UV chain breakage may occur too infrequently to be of biological importance.

DNA-DNA Crosslinks

DNA-DNA crosslinks leading to gel formation have been observed in DNA irradiated while dry^(33,34) and in UV-irradiated salmon sperm heads⁽³⁵⁾ where the DNA is known to be very tightly packed, but have *not* been observed in UV irradiated wet cells⁽³⁵⁾. It is interesting therefore that although pyrimidine dimers are suspected of being involved in the formation of DNA-DNA crosslinks⁽³⁴⁾ the conditions that favor their formation are conditions that do not favor the formation of the cyclobutane-type thymine dimers.^(36,37) DNA-DNA crosslinks appear to be of little biological importance to normal wet cells but may achieve a position of greater biological importance when cells, viruses or transforming DNA are irradiated dry.

Another type of DNA-DNA crosslinking causes the two strands of a single molecule of DNA to be crosslinked so that they can no longer be made to separate completely when treated with heat or formamide.⁽³⁸⁾ For a given dose of UV the extent of crosslinking increased pro-

portionally with the adenine–thymine content of the DNA samples, suggesting that some type of dimer of thymine might be responsible for the crosslinking;⁽³¹⁾ however, the chemical nature of these crosslinks is still unknown. DNA in which almost all of the thymine was replaced by bromouracil was about five times more sensitive to interstrand crosslinking by UV than was normal DNA.⁽³⁹⁾ Since no interchain crosslinks were detected in phage T7 irradiated to a survival of 1%⁽³²⁾ the biological importance of this lesion seems in doubt at low doses of UV. However, this lesion may achieve a position of greater biological importance at higher doses of UV in those cells that are relatively resistant to ultraviolet radiation.

The Crosslinking of DNA to Protein

There is a progressive decrease in the amount of DNA that can be extracted with detergent from bacteria (and other cells) following increasing doses of UV.^(35,40) The DNA that was lost from the soluble phase due to irradiation could be quantitatively accounted for in the precipitate containing the denatured proteins. Treatment of this material with trypsin, however, yielded free DNA. These data suggest that the DNA was crosslinked to protein. Further proof came from experiments showing that DNA and protein could be crosslinked *in vitro*^(5,40,41) (Fig. 3).

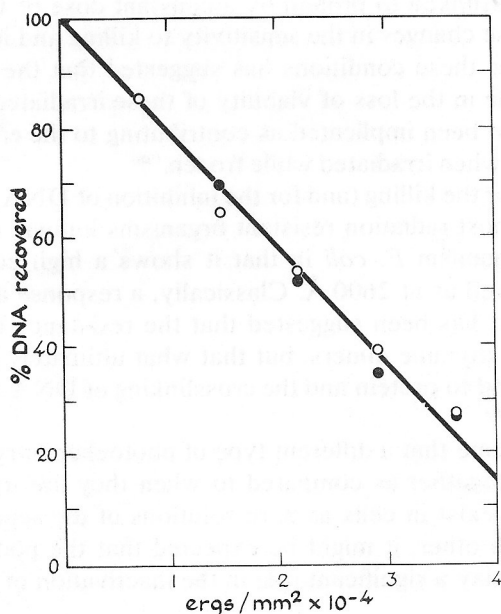


FIG. 3. The photochemical crosslinking of DNA and protein *in vitro*. Solutions containing 10 mg bovine serum albumin and 0.03 mg DNA-thymine-2-C-14 (8.85×10^4 cpm/OD₂₆₀ unit) in 4.2 ml H₂O were irradiated (2537 Å) for various times and aliquots were processed for the recovery of DNA.⁽⁴⁰⁾ It had been previously shown that there is no loss of DNA when irradiated in the absence of protein.^(5,41)

The precise chemical mechanism by which DNA and protein are crosslinked is not known; however, the recent isolation of a dimer of uracil and cysteine (5-S-cysteine, 6-hydrouracil) (Fig. 4) from the *in vitro* UV irradiation of a solution of uracil and cysteine may serve as a possible model for the crosslinking phenomenon.⁽⁴²⁾ The photochemical addition of cysteine-S-35

to poly-U, poly-C, poly-T, RNA and DNA has also been demonstrated.⁽⁴³⁾ The presence of cysteine markedly inhibits the *in vitro* photochemical crosslinking of *E. coli* DNA and bovine serum albumin presumably by competing with the amino acid residues on the protein for attachment to the cytosine residues of the DNA.⁽⁴⁴⁾

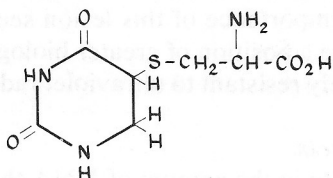


FIG. 4. 5-S-Cysteine, 6-hydrouracil.

The biological importance of this DNA-protein crosslinking phenomenon has been indicated by studies in which the intrinsic sensitivity of cells to killing by UV has been changed by growth in selected nutritionally deficient media and these changes have been shown to be accompanied by a similar directional and time sequential change in the intrinsic sensitivity of the DNA to become crosslinked to protein by a constant dose of UV.^(41,45) The near equivalence in the timing of these changes in the sensitivity to killing and in the crosslinking of DNA and protein by UV under these conditions has suggested that the crosslinking phenomenon must play a significant role in the loss of viability of these irradiated cells. The crosslinking of DNA and protein has also been implicated as contributing to the enhanced sensitivity of cells of *E. coli* to killing by UV when irradiated while frozen.⁽³⁰⁾

The action spectrum for the killing (and for the inhibition of DNA synthesis) of *Micrococcus radiodurans*, one of the most radiation resistant organisms known, differs markedly from that for the more sensitive organism *E. coli* in that it shows a high component of sensitivity to irradiation at 2800 Å as well as at 2600 Å. Classically, a response at 2800 Å has indicated an involvement of protein. It has been suggested that the resistance of this organism to UV is due to its ability to repair thymine dimers, but that what ultimately kills the organism is some sort of damage to DNA and to protein and the crosslinking of DNA and protein may constitute one type of such damage.⁽⁴⁶⁾

It is reasonable to assume that a different type of photochemistry might arise when protein and DNA are irradiated together as compared to when they are irradiated separately. Since DNA and protein do not exist in cells as pure solutions of the separate molecules but are in intimate contact with each other, it might be expected that the photochemical interaction of DNA and protein would play a significant role in the inactivation of UV irradiated cells under certain conditions.

The Effect of Base Composition on the Intrinsic Sensitivity of DNA to UV

Different genetic markers of transforming DNA with different base compositions have been shown to differ in their sensitivity to UV inactivation.⁽⁴⁷⁾ This latter result is in harmony with the observation that there is a correlation between the base composition of the DNA of a particular bacterial strain and its radiation sensitivity. Thus, as the adenine-thymine content increased (G-C decreased) the cells showed an increased sensitivity to killing by UV.^(48,49) This relationship would seem to be adequately explained by our present knowledge of the importance of thymine photoproducts in the UV inactivation of DNA.

The Effect of Substitution by Halogenated Pyrimidines on the Intrinsic Sensitivity of DNA to UV

The intrinsic sensitivity of DNA can be altered by replacing the thymine residues by halogen substituted analogs. Cells that have incorporated halogenated analogs of thymine into their DNA are much more sensitive to killing by UV.⁽⁵⁰⁾ For a given dose of UV, the bromouracil in the DNA of these cells is about twice as sensitive to photochemical alteration as is thymine.⁽⁵¹⁾ This alteration in the intrinsic sensitivity of the DNA must certainly contribute significantly to the altered radiation sensitivity of these cells.

Cells containing bromouracil-substituted DNA show a five-fold greater sensitivity to UV crosslinking with protein than normal cells.⁽⁵⁾ Irradiated DNA containing bromouracil exhibits a greater sensitivity to intramolecular-interstrand crosslinking,⁽³⁹⁾ and is more susceptible to a UV-induced decrease in sedimentation coefficient.^(31,39)

This alteration in the intrinsic sensitivity of the DNA by analog substitution, however, is not the only manifestation of the altered radiation sensitivity of analog-substituted cells. Irradiated bacteriophage that is substituted with bromouracil cannot be photoreactivated nor can it be dark reactivated.^(52,53) The photoproducts of bromouracil therefore do not seem to be amenable to repair by currently known mechanisms.

The increased sensitivity of halogenated pyrimidine-substituted DNA *in vivo* and *in vitro* can be explained both on the basis that these analogs show an increased sensitivity to photochemical alteration and thus increase the intrinsic sensitivity of the DNA, and on the fact that the photoproducts produced seem to be refractory to repair.

The Effect of the Environment During Irradiation on the Intrinsic Sensitivity of DNA to UV

The photochemical reactivity of thymine is markedly different if it is irradiated in solution, in frozen solution, or in dry films.⁽⁵⁾ Bromouracil is inert to UV if irradiated in frozen solution^(54,55) unless additional compounds are added.⁽⁵⁴⁾

This extreme importance of the environment on the photochemistry of the bases is also carried over to the photochemistry of DNA and to the sensitivity of cells to irradiation. The formation of the cyclobutane-type thymine dimer is greatly depressed if the DNA is irradiated dry.^(36,37) Bacteriophage T1 cannot be photoreactivated if irradiated dry.^(37,56) Little or no cyclobutane-type thymine dimers are formed in irradiated spores, yet they are the major photoproduct produced in irradiated vegetative cells.^(36,57) The DNA within spores is thought to be dry and spores are more resistant to killing by UV than are vegetative cells. Cells that have been irradiated while frozen are much more sensitive to killing by UV.^(30,58,59) Under these conditions the yield of thymine dimers is greatly depressed but there is an increase in the amount of DNA crosslinked to protein.^(30,59)

Coupled with the effects of the environment on the photochemistry of DNA are the effects of the physical state of the DNA itself upon its susceptibility to UV alteration. The rate of thymine dimer formation in denatured DNA, for example, is about twice that for native DNA.⁽⁶⁰⁾

SUMMARY

Some of the biological effects of UV irradiation can now be explained in terms of specific chemical and physical changes produced in DNA.

Photochemical events in the carbohydrate and purine residues of DNA would appear to

occur too infrequently to be of major biological importance but this point has not been adequately investigated.

Pyrimidine hydrates do not seem to be formed in double-stranded DNA but are formed in single-stranded DNA. The possible importance of hydrates in causing mutations has been demonstrated.

Cyclobutane-type dimers are formed by the pyrimidines, separately and as mixed dimers. The biological importance of this type of dimer has been demonstrated in certain situations. This type of photoproduct is not formed in DNA under all conditions so it cannot occupy a position of supreme biological importance in all situations. Other types of photoproducts therefore must also be of biological importance.

The biological importance of DNA-protein crosslinking at low doses of UV has been demonstrated under certain conditions. One chemical mechanism for this crosslinking may involve the attachment of amino acid residues through their SH (or OH) groups to the 5 (or 6) carbon of cytosine and/or thymine.

UV irradiation causes chain breakage and the formation of DNA-DNA crosslinks but these usually occur only at high doses so that their importance at low doses seems questionable.

The intrinsic sensitivity of DNA to alteration by UV can be affected by a change in base composition, substitution by analogs and by altering the environment and physical state of the DNA during irradiation.

Although a given photochemical lesion has been shown to be of biological importance under certain conditions it is not expected that it should enjoy similar importance under all conditions. The absolute biological importance of any photoproduct depends upon (1) whether or not it is formed under a particular set of experimental conditions and (2) if formed, whether or not the particular system under study is capable of repairing the lesion.

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