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REACTION SELECTIVITY AND MOLECULAR ASSOCIATION IN PHOTOCHEMICAL REACTIONS OF NUCLEIC ACIDS AND THEIR CONSTITUENTS

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Abstract - The light-induced free radical reactions of purine and pyrimidine bases, nucleosides, and nucleotides with a variety of substrates have been found to be selective for the purines. The selectivity has been shown to result from the suppression of the reactivity of the pyrimidines due to the presence of the purines. It is suggested that base stacking is responsible for this effect. It has been established that steric hindrance imposed by the phosphate group of nucleotides determines the reactivity of adenosine moieties. The photoalkylation of DNA with alcohols or amines leads to selective modification of the purine moieties. Specific covalent cross-linking of RNase with its competitive inhibitors pUp or pCp occurs upon irradiation of the complexes.

INTRODUCTION

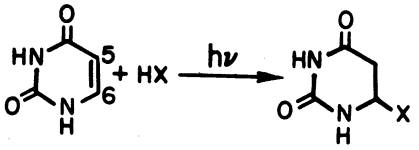
The damaging effects of ultraviolet radiation on living organisms has been realized long ago. These include inactivation of viruses, bacteria, induction of mutations, enhancement of genetic recombination, chromosomal aberrations, and initiation of malignant growth. While the damaging effects of ultraviolet radiation have been known for a long time, only recently has the photochemical basis of many of these effects been elucidated. For example, some of the biological effects of radiation can now be explained in terms of specific chemical and physical changes in nucleic acids. It has been shown that ultraviolet light irradiation of DNA *in vitro* and *in vivo* results in the dimerization of adjacent pyrimidine moieties in the polynucleotide chain. These cyclobutane pyrimidine dimers have been studied extensively and most of the evidence indicates that they are responsible for a major part of the lethal and mutagenic action of ultraviolet light radiation (Ref. 1). The effects observed in higher organisms can be explained in similar terms; thus, thymine dimers have been implicated as being responsible for skin cancer. However, thymine dimer formation is not the only photochemical modification produced in living systems. Crosslinks between proteins and DNA and chain-breakage in DNA have been recognized as resulting from the irradiation of living systems (Ref. 2).

The recent study of the photochemistry of nucleic acid constituents concentrated mainly on the reactions of the pyrimidines (Ref. 1). The major photoproducts identified were the cyclobutane-type dimers, and photosensitization, mainly with ketonic photosensitizers, was utilized for the selective production of these dimers in nucleic acids (Ref. 3). This direction of research has been consistent with the notion that the pyrimidine bases are the light-sensitive sites in nucleic acids. It has, however, been shown recently that purines also undergo light-induced reactions. These involve free radical intermediates and result in the substitution of the appropriate moiety for the H-8 atom of the purine (Ref. 4-7). The multiplicity of products thus formed in irradiated DNA complicates isolation and chemical identification of photoproducts, and further interferes with the correlation between a given photoproduct and the accompanying biological effect. The induction of selective photochemical modifications in one of the constituents of the nucleic acid can serve as a most powerful tool for the study of this correlation. Furthermore, the elucidation of the origin of this selectivity might shed light on structure and conformation of polynucleotides.

It has been the aim of the described research to examine the selectivity of light-induced reactions for the various constituents of nucleic acids, and eventually develop selective reactions for purines and purine moieties of the biopolymers.

PHOTOALKYLATION OF PYRIMIDINES AND PURINES

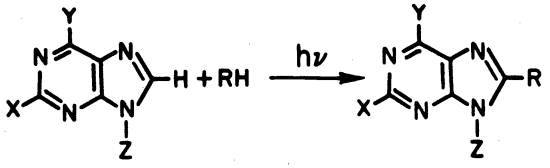
Ultraviolet light irradiation of uracil or cytosine, or their derivatives, with a variety of compounds leads to the formation of the appropriate photoadduct. Thus, a photohydrate of uracil is formed upon irradiation of its aqueous solutions (Ref. 8). Similarly, irradiation of uracil derivatives in the presence of alcohols leads to the formation of photoadducts the structure of which depends upon the conditions of irradiation; two types of photoadducts have been reported, an ether-type adduct and an alcohol-type adduct (Ref. 9). Pyrimidine derivatives, usually those of uracil, undergo heteroadduct formation when irradiated with a variety of other organic compounds, like amines, amino acids, and sulfite ions (Ref. 10).



X = CN, $HNNH_2$, CH_2NH_2, SO_3^-, BH_3

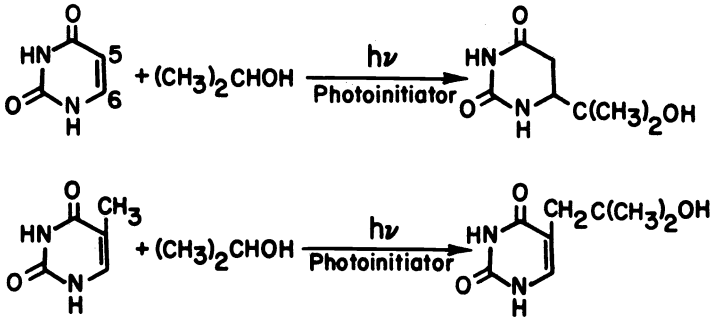
The photochemical reactions of thymine and thymine derivatives with such substrates have been less explored. Substitution of the appropriate moiety for the hydrogen atom at the C-5 methyl group of thymine or, in other cases, addition of the substrate across the 5,6-double bond have beeh observed (Ref. 11).

Less information is available concerning the effect of radiation on purines and purine moieties in nucleic acids. Some ultraviolet light- and γ-ray-induced reactions of purines have been described recently. These include reactions of purines, purine nucleosides and nucleotides with alcohols, amines, ethers, and acetals. With the exception of purine itself, these photoreactions led to the substitution of the appropriate moiety for the hydrogen atom at the C-8 position of the purine system (Ref. 12).



X = H, NH_2; Y = NH_2, OH; Z = H, ribose or deoxyribose
RH = alcohol, amine, amino acid, ether or acetal

These reactions can be induced directly by ultraviolet light (λ>260 nm), which is absorbed by the purine, or indirectly with light of longer wavelength (λ>290 nm) through the use of ketonic photosensitizers or peroxidic photoinitiators. The reactions involve free radical intermediates and, in the case of the peroxidic initiation, proceed through the absorption of the incident light by the peroxide. Excitation of the peroxide is followed by its fragmentation to oxy radicals, which abstract a hydrogen from RH to yield the free radicals. These are subsequently scavenged by the purine to yield the appropriate product. The use of the peroxidic photoinitiation system for the reactions of uracil or thymine derivatives with alcohols led to the exclusive formation of the pyrimidine-alcohol adduct without any formation of cyclobutane-type dimers (Ref. 13).



Selectivity in the light-induced alkylation reactions of purines and pyrimidines
Since the described photoalkylation reactions lead to a single photoproduct of each base, they have been chosen as a suitable system for the study of the selectivity of light-induced free radical reactions of nucleic acid constituents. We have found that when irradiated separatedly, the pyrimidines exhibited a comparable or even higher reactivity than the purines; however, in irradiated mixtures of the bases the purine photoproducts predominated (Ref. 14 & 15). These results are summarized in Table 1.

TABLE 1. Photochemical and γ-ray-induced reactions of mixtures of pyrimidines and purines with 2-propanol

Pyrimidine + Purine	Product %	
	Pyrimidine	Purine
1,3-Dimethyluracil (DMU) + Caffeine	4	60
1,3-Dimethylthymine(DMT) + Caffeine	-	90
Uracil + Adenine	37	35
Thymine + Adenine	3	82
Thymine + Adenine	-	75
Thymidine + Adenosine	-	98
Uridine + Adenosine	5	60
Thymidine + Guanosine	3	78
TMP + AMP	7	46

The selectivity of the reactions was shown to result from the suppression of the reactivity of the pyrimidines due to the presence of the purines. This was evidenced by comparison of quantum yields for product formation when the bases reacted separatedly to those in the mixtures. Thus, the quantum yields for the formation of the pyrimidine-alcohol photoadducts were usually higher than those of the purine-alcohol photoproduct when irradiated separatedly. In equimolar mixtures of pyrimidines and purines, the formation of the pyrimidine product was inhibited, while that of the purine remained unchanged. This data is summarized in Table 2.

TABLE 2. Quantum yields of product formation of pyrimidines and purines with 2-propanol

Bases		Mixtures		
			Φ	
	Φ	Purine + pyrimidine	Purine	Pyrimidine
DMU	0.076	Caffeine + DMU	0.035	0
DMT	0.022	Caffeine + DMT	0.041	0
Uracil	0.47	Adenine + uracil	0.004	0.004
Caffeine	0.036	Adenosine+ uridine	0.015	0
Adenine	0.004			
Uridine	0.05			
Adenosine	0.015			

It is proposed that heteroassociation of the bases, which is of base stacking nature under these reaction conditions, is responsible for this effect. This assumption is supported by the observation that the degree of suppression of the reactivity of the pyrimidines in the presence of the purines is stronger at higher concentrations of the bases in the mixture, and at lower temperatures. Furthermore, the effect was sensitive to the presence of a variety of anions which either enhance or decrease association, and could also be correlated with osmotic coefficient data.

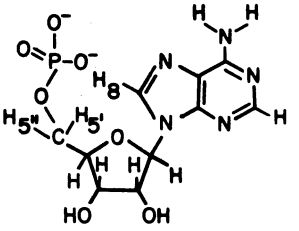
It has been concluded, therefore, that the selectivity of the photoalkylation reactions for the purines in the isolated bases or nucleosides is derived primarily from the suppression of the reactivity of the pyrimidine due to the presence of the purine, and results from the heteroassociation of the bases.

PHOTOALKYLATION OF MONONUCLEOSIDES AND DINUCLEOSIDE MONOPHOSPHATES

The photoalkylation reactions with alcohols were applied to a variety of purine and pyrimidine mononucleotides and dinucleoside monophosphates in order to examine the effect of the phosphate group on the reactivity of the bases and the selectivity of the reactions. It has been found that adenosine moieties blocked at their 3'-hydroxyl group were alkylated faster than those blocked at their 5'-hydroxyl. The reactivity of the uridine moieties of 3'-UMP,5'-UMP, and uridylyl-(3',5')-uridine (U-U) was not affected by the location of the phosphate group. The uridine moiety of uridylyl-(3',5')-adenosine (U-A) was modified, however, faster than that of adenylyl-(3',5')-uridine (A-U). Cytidine and cytidine moieties were unreactive under the reaction conditions (Ref. 16).

The results of this study indicate that the 8-position of the adenine moiety of 3'-AMP is more exposed to an attack by free radicals than that of 5'-AMP. A similar effect operates in adenine-containing dinucleoside monophosphates, where an adenosine blocked at its 3'-hydroxyl is more exposed to an attack than a 5'-blocked adenosine. Our results further indicate that the 5,6-double bond of the uracil moiety in 3'-UMP is nearly as equally exposed to an attack by the free radicals as that of 5'-UMP. The same effect operates in U-U, where both uridine moieties are exposed to the same extent to an attack by the ketyl radicals. The uridine moiety of U-A is more exposed than that of A-U.

The reactions were carried out in very dilute solutions, thus eliminating the possibility of intermolecular base stacking. Therefore, the reactivities of the adenosine and uridine moieties in the mononucleotides have to be evaluated in terms other than base stacking. It is,therefore, concluded that the reactivity of the adenosine moieties depends primarily on steric factors imposed by the phosphate group. This conclusion is in agreement with that derived from nmr data, which assign the *anti* conformation to 5'-AMP in solution. In this conformation the phosphate group is in close proximity to the 8-position of the adenine, as implied by the deshielding effect of the phosphate on the H-8 proton of the purine. Such an interaction does not exist in 3'-AMP (Ref. 17).



5'-AMP

In adenylyl-(3',5')-adenosine (A-A), the adenosine moiety linked through the 3'-hydroxyl was modified faster than that linked through the 5'-hydroxyl. Since A-A in solution assumes the *anti* conformation (Ref. 17), the phosphate group is in close proximity to the 8-position of the adenosine which is linked through the 5'-hydroxyl. Although base stacking exists in the molecule, it has been found that it does not affect the reactivities of the adenosine moieties. Thus, here too, the difference in the reactivities of the adenosine moieties results from steric hindrance imposed by the phosphate group, and it is effective on the 8-position of the adenosine moiety which is blocked at the 5'-hydroxyl. A similar effect has been observed in all the adenosine ribodinucleoside monophosphates.

Since cytidine moieties are inert to these photoalkylation reactions and thymine moieties react rather slowly, the reactions of adenine-cytosine or adenine-thymine systems are highly selective for the adenine moieties, irrespective of their location in the dinucleoside monophosphate.

The selectivity in the adenine-uracil system presents a different case, since uracil, as compared with other pyrimidines, exhibits a higher reactivity in the reported reactions. Thus, while 3'-AMP and 5'-UMP are alkylated with comparable rates, the rate of modification of the adenosine of A-U is nearly eight-fold faster than that of the uridine moiety. On the other hand, 3'-UMP reacts nearly twice as fast as 5'-AMP, and this preference of the reaction for the uridine moiety is preserved in U-A. It is assumed that the difference in the reactivity of the uracil moiety of A-U as compared with that of U-A, results from intramolecular stacking of the bases which appears to be stronger in A-U. This conclusion is in agreement with previously reported nmr data (Ref. 17 & 18), which indicate that the 5,6-double bond of uridine in A-U is in close proximity to the adenosine moiety, while in U-A only partial overlap of the uridine and adenosine is possible. It has further been shown that the presence of $(NH_4)_2SO_4$, which is known to enhance stacking in nucleotides (Ref. 19), supresses considerably the reactivity of uridine of U-A, thus reversing the selectivity of the reaction in favor of adenosine.

Based on these results, it is tentatively concluded that the reactivity of adenosine moieties in light-induced and free radical reactions, involving the 8-position of the purine, is affected by the location of the sugar-phosphate linkage. This feature and intramolecular base

stacking determine the degree of selectivity of these reactions for the appropriate base in systems containing adenine and uracil (Ref. 16).

PHOTOALKYLATION OF POLYNUCLEOTIDES

The photoalkylation reactions were also applied to purine and pyrimidine polynucleotides. When irradiated (λ>290 nm) with alcohols in the presence of $(Bu^tO)_2$, poly(A) or poly(U) were modified in high yields (>90% of the residues). Poly(A), however, was modified with a slower rate as compared with that of poly(U). We used mixtures of poly(U) and AMP, or poly(A) and UMP for the study of the possible suppression of reactivity of the pyrimidines in these systems, i.e. a polymer and a monomer unit. Our results showed that protection of poly(U) by AMP took place, while no protection of UMP by poly(A) occurred (Ref. 15). The amount of protection of the pyrimidine moiety by the purine is defined as the ratio between the percent of modification of the purine and that of the pyrimidine in the mixture (R_M), divided by the ratio of modification of the same components when irradiated separatedly (R_B), under identical conditions. An R_M/R_B = 3.8 was obtained for the AMP:poly(U) system, while R_M/R_B=1.05 was found for the UMP:poly(A) system. These data indicate that a certain interaction exists between UMP and poly(A), while there is no indication for a similar interaction between UMP and poly(A) (Ref. 17). It has been shown previously that complexation due to the interaction of a polynucleotide with a monomeric unit can only occur when the monomer is a purine derivative (Ref. 17 & 20).

Enzymatic digestion of modified polynucleotides

The application of these reactions to natural biopolymers raises the question whether enzymes involved in nucleic acid functions will recognize the modifications. Initially, we were concerned whether the phospodiester bonds of the modified nucleotides are split by the appropriate enzymes in comparable rates with those of the non-modified residues. For this purpose we irradiated Poly(G), poly(U_{20}G), and poly(A) with 2-propanol or D-ribose and subsequently digested the irradiated polynucleotide with T_1-, pancreatic-, and T_2-RNase, respectively. Results of this research indicate that the modified polynucleotides are sensitive to RNase; however, in some cases the intermediate cyclic modified mononucleotide is produced either exclusively or together with the open form of the mononucleotide (Ref. 21). It is possible that the 8-modified purine moieties are still as good a substrate for the nucleases as the non-modified moieties; they differ only, if at all, in the rates of the splitting of the cyclic phosphate intermediate.

PHOTOALKYLATION OF PURINES IN DNA

The study of the photochemical reactions of the various constituents of nucleic acids in the presence of alcohols or amines, can serve as a useful tool for understanding the effect of the environment of the nucleic acid on the type and ratios of the photoproducts formed. In addition, the design of reaction procedures leading to selective photochemical modifications of nucleic acids might greatly facilitate the investigation of the biological consequences of irradiation. The photoalkylation of DNA with 2-propanol resulted in the substitution of the α-hydroxy*iso*propyl group for the H-8 atom both in adenine and guanine moieties. When acetone was used as a photosensitizer, pyrimidine dimers were formed in the irradiated nucleic acid together with the 8-alkyl derivatives of adenine and guanine (Ref. 22), whereas with $(Bu^tO)_2$ as a photoinitiator, photoalkylation of the purines occurred exclusively (Ref. 23). No pyrimidine photoproducts were observed in the irradiated DNA, however, in denatured DNA a thymine-alcohol adduct was produced. It has further been noticed that product formation is exponentially dependent on time. Since chain-breakage took place during irradiation, it is assumed that these breaks and local melting of hydrogen bonds ease the access of the free radicals to the purine moieties, thus increasing the formation of purine photoproducts.

The reaction of DNA with 2-propylamine in the presence of $(Bu^tO)_2$ has been investigated recently. The reaction resulted in the selective production of 8-α-amino*iso*propyladenine and 8-α-amino*iso*propylguanine, without any formation of pyrimidine photoproducts (Ref. 24).

The adenine and guanine moieties of DNA are photoalkylated to the same extent in these reactions. This differs from the alkylation by chemical alkylating agents which react preferentially with guanine at the N-7 position. This difference results, probably, from the different mechanisms operating, the photochemical alkylation proceeding through free radical intermediates, whereas the chemical alkylation involving polar intermediates (Ref. 25).

NUCLEIC ACID-PROTEIN PHOTO-INTERACTION

The understanding of the molecular basis of the interaction between nucleic acids and proteins is of considerable importance for the proper evaluation of the radiation damage in mammalian cells. This is particularly true since the genetic material of mammalian cells is associated with proteins whose participation in the photochemical reaction may be of critical significance. Indeed, it has been shown that ultraviolet light induces crosslinking between DNA and proteins *in vivo* and *in vitro*, and that the presence of proteins has a pronounced effect on the distribution and type of photoproducts formed in DNA (Ref. 2). On the other hand, photochemistry can serve as a tool for the study of nucleic acid-protein interactions by "freezing" existing interactions in the complexes; since upon exposure of the composure of the nucleic acid-protein complexes to ultraviolet irradiation covalent bonds are formed which link together the two partners in the complex (Ref. 26). The potential utility of this approach depends on whether covalent bonds are formed specifically between residues which are in close proximity at the binding sites of native complexes.

Sperling and Havron (Ref. 27) have tested the complexes formed between RNase and its competitive inhibitors cytidine-2'(3'),5'-diphosphate (pCp), and uridine-2'(3'),5'-diphosphate (pUp). Irradiation of both complexes in the presence of acetone as a photosensitizer resulted in covalent bond formation between the pyrimidine nucleotides and the enzyme. Tryptic digestion and chromatographic analysis of the peptides revealed a single and specific peptide which became covalently linked to both nucleotide inhibitors. The amino acid composition of this peptide was consistent with the sequence Asn 67 - Arg 85 of RNase. The specificity of crosslinking has further been established by showing that (i) denatured RNase failed to crosslink with either inhibitors; (ii) the addition of increasing concentration of "cold" 3'-UMP inhibited completely the incorporation of radioactive pUp or pCp into the enzyme; (iii) there has been no non-specific crosslinking with larger excess of the inhibitor.

Sperling (Ref. 28) has further shown that a covalent crosslink is formed between histone H4 and the adenine moiety of ATP, when the complex they form is irradiated with ultraviolet light of λ>290 nm in the presence of acetone. A yield of nearly 50% of crosslinked product was thus obtained.

CONCLUSION

A series of light-induced free radical reactions of purines with alcohols, amines, amino acids, ethers or acetals has been developed recently. The reactions result in the substitution of the appropriate group for the H-8 atom of the purine. In contrast to the general notion that pyrimidines are more susceptible to light-induced transformations,it has been found that in many cases purine susceptibility predominates. The observed "enhanced" reactivity of the purines has been shown to result from the suppression of the reactivity of the pyrimidine due to the presence of the purine. It is proposed that this phenomenon, already operating at the molecular level of the bases or their nucleosides, results from base stacking. These reactions can serve as a handle by which both purine and pyrimidine ends in heteroassociates can be observed simultaneously, and a great deal of information regarding heteroassociates of polynucleotides can be derived from the chemical reactivity of the monomer units in the appropriate associates.

This data presents a step towards the development of a series of selective reactions for the purine moieties of nucleic acids. The elucidation of the origin of this selectivity might shed light on the structure and conformation of polynucleotides and their behavior in light-induced reactions. This data further emphasizes the role that purines play in photochemical trnasformations of nucleic acids, including crosslinking with proteins.

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