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THE PHOTOCHEMICAL ADDITION OF AMINO ACIDS AND PROTEINS TO NUCLEIC ACIDS

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ABSTRACT: When bacterial cells are irradiated with ultraviolet light, DNA and protein become photochemically cross-linked. This photochemical reaction appears to be the major lethal lesion in irradiated bacteria under certain experimental conditions. We have turned to *in vitro* model systems in order to gain some insight into the mechanism(s) by which DNA and protein are photochemically cross-linked *in vivo*. A survey was performed of the ability of the 22 common amino acids to add photochemically (254 nm) to ^{14}C-uracil. The 11 reactive amino acids were glycine, serine, phenylalanine, tyrosine, tryptophan, cystine, cysteine, methionine, histidine, arginine and lysine. The most reactive amino acids were phenylalanine, tyrosine and cysteine. We have isolated the cysteine adduct and have shown it to be 5-S-cysteine-6-hydrouracil. The analogous thymine adduct has also been isolated and its tentative structure is 5-S-cysteine-6-hydrothymine. We have studied the kinetics of the photochemical addition of ^{35}S-cysteine to various synthetic and natural polynucleotides. Preliminary data on the tyrosine adducts to uracil are reported.

INTRODUCTION

When bacterial cells are irradiated with ultraviolet light, there is a dose-dependent decrease in the amount of deoxyribonucleic acid (DNA) that can be isolated in pure form from these irradiated bacteria (1, 2). This phenomenon arises as a consequence of the photochemical cross-linking of the DNA with protein. This cross-linking of DNA and protein appears to be the major lethal lesion in irradiated bacteria under certain experimental conditions.

For example, the enhanced yield of DNA cross-linked to protein for a given dose of ultraviolet radiation may explain the enhanced sensitivity of bacteria to killing by UV under conditions of thymine starvation (3) or when cells are irradiated while frozen (4). The chemical mechanism by which DNA and protein are photochemically cross-linked in vivo is not known. Because of the complexities of dealing with cellular systems, we have turned to in vitro model systems in order to study the possible mechanisms by which DNA and protein may become photochemically cross-linked.

EXPERIMENTAL

The two photochemical reactions of the pyrimidines that are best understood are the formation of cyclobutane-type dimers through carbon atoms 5 and 6 and the hydration reaction which involves the addition of a molecule of water across the 5-6 double bond of single pyrimidines (for recent reviews see 5-7). We therefore wondered whether reactions of these types might be involved in the photochemical cross-linking of DNA and protein. We have tried to form cross-adducts between thymine and the several aromatic amino acids by irradiating suitable mixtures in frozen solution; conditions which favor the dimerization of thymine when irradiated by itself. These attempts have thus far proved unsuccessful. We have also irradiated various OH and SH amino acids in solution with uracil in the hope of finding reactions analogous to the addition of water across the 5-6 double bond. Early experiments indicated that the SH amino acid, cysteine, was very reactive in photochemically combining with uracil. We have isolated this photoproduct and determined its structure to be 5-S-cysteine-6-hydrouracil (Figure 1).

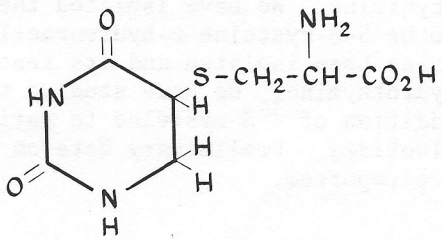

Figure 1. 5-S-cysteine-6-hydrouracil (8).

Ultraviolet, infrared, nuclear magnetic resonance, and mass spectroscopy were used to identify this photoproduct (8). Treating the photoproduct with deuterated Raney nickel yields 5-mono-deuteriodihydrouracil, thus confirming the point of attachment of the cysteine. Raney nickel treatment of the cross-adduct also yields alanine; the desulfurated product of cysteine. 5-S-cysteine-6-hydrouracil-HCl is stable to heat (100°C) in water

solution and is stable to 6 N HCl at room temperature but is not stable to the heat and acid conditions used for the hydrolysis of DNA. It is quite unstable to alkali.

To investigate the scope of the raction of cysteine with the various pyrimidines we have determined the rate constants for the uptake of cysteine by various synthetic and natural polynucleotides per dose of ultraviolet radiation (9). These results are summarized in Table 1.

TABLE I: Rate Constants for the Photochemical Addition of [^{35}S]Cysteine to Polynucleotides.

Polynucleotide	K[a] Exptl	K[a] Calcd
Poly rU	21.8 (13.3)[d]	
Poly rU:rA	0.7[e] (U only)	
Poly rA	0.6	
Poly rC	8.1 (0.6)[d]	
RNA (yeast)[b]	21.8[e]	
	4.8[e]	
Poly dC	2.6	
Poly dC:dG	2.6 (C only)	
Poly dT	5.4	
Poly dT:dA (heated)[c]	4.2 (T only)	
Poly dT:dA	2.6 (T only)	
Poly dAT:dAT	1.1 (T only)	
DNA (calf thymus)	2.6[f]	2.6[g]
DNA (heated)[c]	4.2[f]	4.2[h]

[a] K = (μmoles of cysteine/μmole of PO_4 involved)/ergs/mm^2 $\times$ 10^8 (at pH 5). [b] RNA shows a biphasic uptake of cysteine. [c] For 15 min at 100° in 0.075 M NaCl. Quick cooled. [d] At pH 6.5. [e] For (20% C + 27% U). [f] For (21% C + 29% T). [g] For (dC:dG + dA:dT). [h] For (dC + dT). (9).

Cysteine readily combines with polymers containing uridylic acid, cytidylic acid or thymidylic acid. Whether the polymers are single or double-stranded (and/or protonated) has a profound effect upon

the reaction rate.

Whereas the cysteine that was photochemically combined with polyuridylic acid was completely stable to heating at 65°C for 60 minutes, there was a 16% loss of cysteine from polydeoxythymidylic acid and a 49% loss from polydeoxycytidylic acid. Using these percentage lability figures and correcting for the thymine and cytosine content of the DNA, we calculated that 30% of the cysteine photochemically attached to calf thymus DNA should be heat labile. This was in good agreement with the value of 36% observed experimentally. These results suggest that there are at least two mechanisms for the photochemical linkage of cysteine to cytidylic acid and to thymidylic acid in these polymers. The heat lability of certain of the amino acid adducts to DNA may help to explain some of our problems in dealing with the whole bacterial system where we have heated the cells in detergent at about 65°C in order to lyse the cells and to denature their proteins.

We have isolated the cysteine adduct to thymine, and based upon nuclear magnetic resonance spectroscopy, the structure of this photoproduct appears to be analagous to the uracil product and has been identified as 5-S-cysteine-6-hydrothymine (10).

We have observed that dihydrouracil is formed in significant quantities when irradiated in the presence of SH compounds such as cysteine, cysteamine and H_2S (8). Recently, Jellinek and Johns (11) have undertaken a study of the chemical mechanisms involved in the photochemical addition of cysteine to uracil and in the formation of dihydrouracil. Their data suggest that the triplet excited state of uracil can abstract hydrogen atoms from cysteine to form dihydrouracil. The thiyl radicals generated by this process can add to ground state uracil molecules to yield the cross-adduct between cysteine and uracil.

Two reactions of the pyrimidines that have been described by other authors may have some importance in the photochemical cross-linking of DNA and protein. Alcantara and Wang (12) have observed the formation of 5-formyluracil when thymine in aqueous solution is exposed to ultraviolet radiation. If this reaction occurs in irradiated DNA, the formyl group could then react with a protein amino group to give a covalent bond between DNA and protein (13).

Janion and Shugar (14) have observed that dihydrocytosine will react with glycine such that the amino group of the dihydrocytosine is replaced by the amino group of the glycine, resulting in a covalent link between dihydrocytosine and glycine. Since both the photohydrate and cyclobutane-type dimer of cytosine are analogs of dihydrocytosine, one may predict that the addition of protein amino groups to these cytosine photoproducts might serve as another mechanism by which DNA and protein are cross-linked by ultraviolet

radiation.

Several lines of evidence have suggested that cysteine is probably not the only amino acid capable of combining photochemically with the nucleic acids. Our first line of evidence was the observation that the protein gelatin, which contains no cysteine, does photochemically cross-link with DNA in vitro, albeit at a much reduced efficiency compared with bovine serum albumin which does contain cyst(e)ine (15). In order to determine the scope of the reactivity of amino acids with the nucleic acids we have investigated the ability of the 22 common amino acids to add photochemically (254 nm) to ^{14}C-uracil (16). The 11 reactive amino acids were glycine, serine, phenylalanine, tyrosine, tryptophan, cystine, cysteine, methionine, histidine, arginine and lysine (Figure 2).

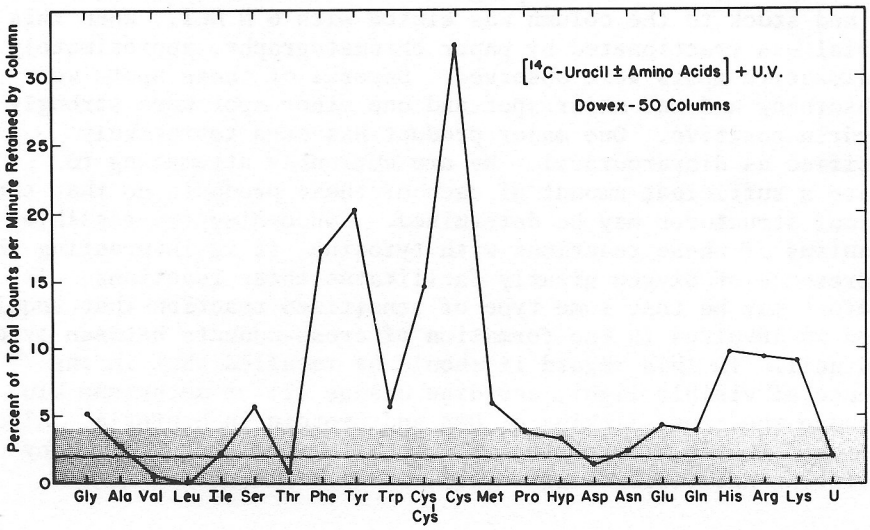

Figure 2. The Photochemical Addition of Amino Acids to ^{14}C-Uracil. A 0.2 ml aliquot of amino acid solution (0.01 M; except tyrosine at 0.003 M) was mixed with 0.05 ml of ^{14}C-2-uracil (0.0011 M; 25 μCi/ml). The molar ratio of amino acid to uracil was thus ≃36:1 (except for tyrosine at ≃11:1). The solution was irradiated for 200 minutes in a Pyrex spot plate in contact with the rim of a Mineralight U.V. lamp (Model UVS-11, Ultra-Violet Products, Inc.) whose output is mainly at 254 nm. An aliquot was then assayed for content of radioactivity (liquid scintillation counter) and 0.05 ml was introduced to a 4 ml column of Dowex-50-HCl in a plastic 5 ml syringe. The column was rinsed with 25-one ml portions of water (into a volumetric flask) and an aliquot of the combined effluent was counted for radioactivity. The 100% sample minus the material that did not stick to the column gives the amount retained by the column. Most of these counts could be recovered by eluting with 6 N HCl. The results plotted here are

the average of 2-3 experiments (and 9 for ^{14}C-uracil (U) alone). The hatched area indicates the spread of the data for ^{14}C-uracil irradiated in the absence of amino acids. (16)

The most reactive amino acids were phenylalanine, tyrosine and cysteine. The finding that 11 amino acids react photochemically with uracil suggests many new mechanisms by which DNA and protein can become cross-linked in vivo by ultraviolet radiation.

Having elucidated the photochemical reaction between cysteine and uracil we are currently investigating the nature of the photochemical reaction between tyrosine and uracil. A mixture of tyrosine and ^{14}C-uracil was irradiated (254 nm) and then poured through an ion exchange column that reacts with basic groups (Dowex 50). The column was purged with water and then the material that had stuck to the column was eluted with 6 N HCl. When this material was fractionated by paper chromatography, approximately 6 radioactive spots were observed. Several of these spots were UV-absorbing and one major spot and one minor spot were strongly ninhydrin positive. One major product has been tentatively identified as dihydrouracil. We are currently attempting to isolate a sufficient amount of each of these products so that their chemical structures may be determined. Concerning the possible mechanisms of these reactions with tyrosine, it is interesting that the presence of oxygen greatly facilitates these reactions. It therefore may be that some type of sensitized reaction that requires oxygen is involved in the formation of cross-adducts between tyrosine and uracil. In this regard it should be recalled that in the presence of visible light, acridine orange (1) or methylene blue (5) cause the cross-linking of DNA and protein in bacteria. Also, the cross-linking of cysteine and uracil has been sensitized by riboflavine and visible light (11).

CONCLUSIONS

In the past, the photochemistry of the nucleic acids and of the proteins have been studied separately. However, in living cells, the nucleic acids and the proteins are not present in separate compartments but are in constant interaction. It thus seems logical that if there are photochemical reactions between proteins and nucleic acids that these interactions may have significant biological consequences in cells that have been irradiated with ultraviolet light. This appears to be the case.

A new class of compounds have been described; cross-adducts of amino acids and pyrimidines. Since about half of the common amino acids readily cross-link photochemically with uracil, the generality of this type of reaction is established. The mechanisms for the addition of these amino acids to uracil must await the

bulk isolation of the products and their chemical identification.

The observation that the nucleic acids can combine photochemically with amino acids suggests that more attention should be given to these addition reactions in attempts to explain the biological effects of ultraviolet radiation, especially when the effects cannot be adequately explained by the known photoproducts produced in pure DNA.

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