PHOTOCHEMICAL ADDITION OF AMINO ACIDS TO THYMINE

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SUMMARY: The ultraviolet light induced photoreactivity of thymine with the twenty two common amino acids has been surveyed. Lysine, arginine, cysteine, and cystine have been found to be reactive in forming heteroadducts. The results suggest that lysine and arginine may be involved in the experimentally observed ultraviolet induced crosslinking of histones to DNA in DNA-histone complexes.

In recent years there has been considerable interest in the chemical nature of the ultraviolet radiation induced crosslinking between DNA and protein (1). Smith (2) isolated an adduct, 5-S-cysteine, 6-hydrothymine, from UV irradiated aqueous solutions containing thymine and cysteine. Varghese (3) later isolated this compound, along with several other adducts, from photolyzed aqueous thymine-cysteine systems. Smith (4) found that bovine serum albumin will bind to DNA under ultraviolet radiation, but that added cysteine greatly inhibited such crosslinking. Presumably the cysteine competes with the side chains of the amino acid residues in the protein for photoexcited heterocyclic bases in the DNA. In support of this hypothesis Smith (5) found that gelatin, which contains no SH groups, crosslinks less efficiently to DNA than serum albumin, but does bind to some extent. This implies that amino acids, other than cysteine, must be reactive toward adduct formation with the heterocyclic bases of DNA. It has also been found that histone (which contains only a small amount of cysteine, and that confined to one histone fraction (6)) can be crosslinked to DNA in DNA-histone complexes (7).

There have been a number of studies of the photo-reactivity of the heterocyclic bases of DNA with amino acid model compounds. Elad and co-workers have studied the photochemistry of thymine with isopropanol (8) and the photo-

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chemistry of the purine bases with alcohols (8) and amines (9). Considerable work has also been done on the photochemistry of uracil (a constituent of RNA) and its analogs toward amino acids and amino acid model compounds. Yang and co-workers (10) found that several heteroadducts were formed in irradiated N,N-1,3-dimethyluracil (DMU)-propylamine systems and that DMU could be bound photochemically to polylysine. Smith (11) isolated 5-S-cysteine, 6-hydrouracil from UV irradiated uracil-cysteine systems. Jellinek and Johns (12) showed that triplet state uracil abstracts hydrogen from cysteine to form dihydrouracil and thyl radicals. These thyl radicals can then add to ground state uracil to set up a chain reaction leading to the heteroadduct. Elad (13) and Jellinek and Johns (12) have studied uracil-isopropanol systems. Elad also studied the photochemistry of the uracil-methionine system in aqueous solution and isolated dihydrouracil as a product. Smith (14) has surveyed the photoreactivity of the twenty two common amino acids toward heteroadduct formation with uracil and found eleven to be significantly reactive.

In our laboratory we are interested in the nature of photoinduced crosslinking between histone and DNA. We have therefore surveyed the reactivity of the common amino acids toward heteroadduct formation with thymine. The results of this study are reported here. We have used basically the procedure of Smith (14). As a check on the validity of our experimental techniques we initially repeated Smith's work on uracil. His results, along with ours, are presented in Figure 1. As can be seen, the reactivity profiles obtained in the two sets of experiments are quite similar. Figure 2 presents the results obtained with thymine. As compared to uracil, thymine is much less reactive towards adduct formation with the amino acids. Only cysteine, cystine, and the two basic amino acids, lysine and arginine, are significantly reactive, even when the photolysis time is extended to seven hours. The small amount of reaction between methionine and thymine after seven hours is likely due to reaction with mercaptan or disulfide products produced from photolyzed methionine. The color and mercaptan-like odor of the photolyzed

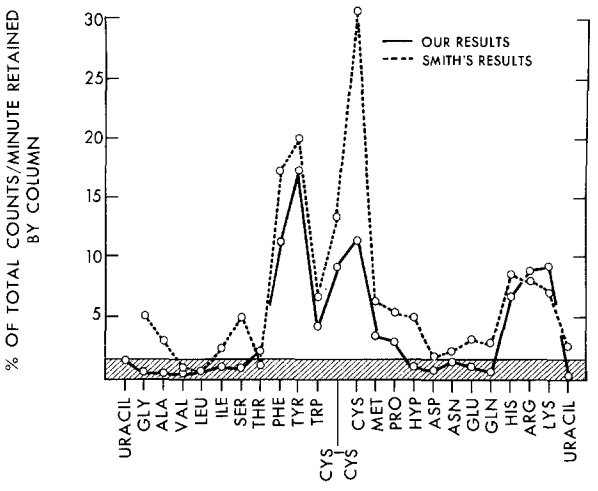

Figure 1

The photochemical addition of amino acids to [^{14}C] uracil. A 1.0 ml aliquot of amino acid solution (0.01 M; except tyrosine at 0.003 M) was mixed with a 1.0 ml aliquot of [^{14}C] uracil (0.0011 M; 5μCi/ml). The molar ratio of amino acid to uracil was thus 9:1 (except for tyrosine at 3:1). The solutions were irradiated for 3.0 hours in plastic ice cube trays (cooled in ice water to retard evaporation) using a General Electric germicidal lamp (Model G 15T8) equipped with a vycor sleeve, thus limiting output to primarily 2537 Å. A .05 ml aliquot was assayed for content of radioactivity (liquid scintillation counter) and another .05 ml was introduced into a prefilled Dowex AG50W-X8 column (Bio-Rad # 7316213). The column was eluted with 25 ml of water, and a 1 ml aliquot of the combined effluents was counted for radioactivity. The 100% sample minus the material that is eluted gives the amount that is retained by the column. Most of the retained counts could be recovered by eluting with 6N HCl. The hatched area gives the spread for [^{14}C] uracil alone (three trials).

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thymine-methionine solution was similar to that obtained when methionine alone in aqueous solution was photolyzed at 2537 A. Tryptophan and tyrosine also appear to be slightly reactive after seven hours, but not after three and one-half hours. In any event the reactivity of these amino acids is low

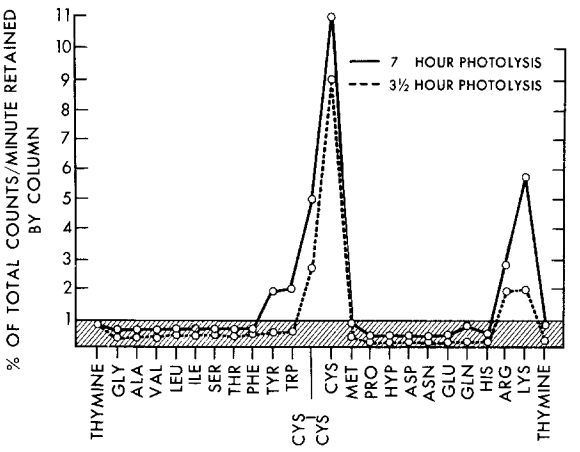

Figure 2

The photochemical addition of amino acids to [^{14}C] thymine.

The experimental conditions are the same as those used in the [^{14}C] uracil experiment. The photolysis times were 3.5 and 7 hours. The data plotted for the 7 hour experiment are the averages of two runs. The shaded area indicates the spread of the data for [^{14}C] thymine alone, (average of four runs).

compared to the reactivity of arginine, lysine, cysteine, and cystine.

Since histone contains very little cysteine or cystine, the above results indicate that thymine-lysine and thymine-arginine adducts may account, at least in part, for the observed crosslinking in irradiated DNA-histone complexes (7). Lysine and arginine are two of the main constituents of histone (6) and, according to current models for DNA-histone interactions, are probably intimately involved in binding histone to the DNA double helix. Thus ample opportunity should exist for excited thymine within a DNA-histone complex to react with these amino acid residues.

Smith and Muen (15), in preliminary results, found that tyrosine was quite reactive and serine and threonine moderately reactive toward uptake by DNA in irradiated DNA-amino acid systems. As these compounds are not

significantly reactive toward thymine, they presumably must be reactive toward one of the other heterocyclic bases of DNA. This hypothesis is supported by the results of Elad and co-workers (8) who report that adenine is much more reactive toward adduct formation with isopropanol, a threonine model, than is thymine. We are currently surveying the reactivity of adenine, guanine, and cytosine toward the common amino acids so as to gain a balanced picture of the reactivity of the amino acids toward heteroadduct formation with the heterocyclic bases of DNA.

ACKNOWLEDGEMENTS

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