FORMATION OF THYMINE-LYSINE ADDUCTS IN IRRADIATED DNA-LYSINE SYSTEMS

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SUMMARY: Thymine, contained in DNA excited by ultraviolet light ($\lambda > 260$ nm), adds lysine to form an acid stable thymine-lysine adduct.

In recent years it has become evident that photochemical cross-linking of proteins to DNA is an important process when systems containing both protein and DNA are irradiated with ultraviolet light (1). It seems likely that this type of cross-linking must be considered if a full understanding of the nature of photo-induced damage to such DNA-protein systems as chromatin in eucaryotic cells is to be achieved. For example, Todd and co-workers (2) have presented evidence that indicates DNA and histones may be cross-linked by ultraviolet light in cultured mammalian cells to form macromolecular adducts.

The chemical nature of photo-induced cross-linking (i.e., which amino acids and which DNA bases are involved, along with the question of adduct structure) is a subject of considerable importance. Varghese and Rauth (3) showed that thymine-cysteine adducts were formed in DNA irradiated in the presence of cysteine and in irradiated mammalian cells: the yield of adduct amounted to about ten percent of that of thymine dimers. While cysteine is not a major component of histone (it accounts for one residue in one histone fraction (4)),

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it is contained in other proteins found along with chromatin in the nucleus (5). Smith (6) has shown that DNA, irradiated in the presence of radioactive labeled amino acids, takes up tyrosine, serine, and threonine as well as cysteine, but did not determine which bases were involved.

Studies have also been reported in which amino acid analogs have been found to add to DNA. Elad and co-workers (7) have shown that adenine and guanine, contained in DNA, react with isopropanol, a threonine side chain analog, either when the DNA is directly excited or in the presence of radical reaction photo-initiators (acetone or di-t-butyl peroxide). In the presence of the photo-initiators a thymine-isopropanol adduct was found as well.

Recently, Schott and Shetlar (8) showed that the DNA base thymine was quite reactive toward photoaddition of four amino acids (lysine, arginine, cysteine, and cystine) and marginally reactive toward addition of tyrosine and tryptophan. We now report evidence that lysine adds to thymine contained in photo-excited DNA to form an acid stable linkage. Such photochemical linkage of lysine, contained in protein, to thymine may be important in cross-linking DNA to histone; lysine is one of the major constitutents of the various histone fractions (4).

Figure 1 shows the results obtained, upon paper electrophoresis, when tritiated thymidine is irradiated in the presence of lysine, and then hydrolyzed with acid. (Thymidine, rather than thymine, was chosen for this study because of its solubility and because the pyrimidine ring, being substituted in the N-1 position, probably behaves more like the base incorporated into DNA.) Material found in strips B and E contains radioactivity and gives a positive ninhydrin test. When the irradiated solution was passed through a a weak acid cation exchange column (IRC-50) lysine was retained, but the products found in strips B and E were not. When this eluate was passed through a strong acid cation exchange column the ninhydrin positive products in strips B and E were retained; they could be recovered by washing the column with HCl. Thymidine was not retained by either column. Control solutions, which either

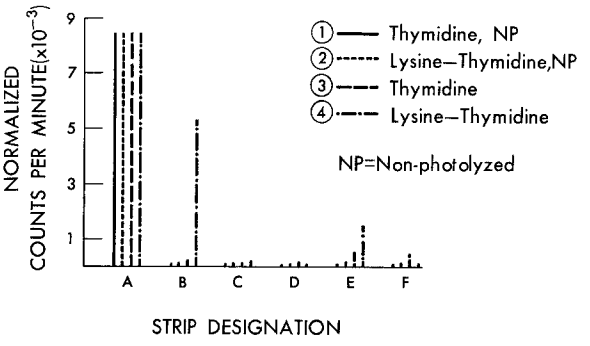

Figure 1

The photochemical addition of lysine to thymidine. A typical experimental protocol is as follows. A 0.2 ml sample of aqueous solution containing 1.52 mg (0.05 mole/liter) of lysine and 1 μCi thymidine (tritium labeled on the 5-methyl; specific activity 7 Ci/mole) was placed in a quartz NMR tube and irradiated with a Hanovia 450 watt medium pressure Hg lamp equipped with a Corex filter (wavelengths passed: $\lambda > 260$ nm) for 36 hours. The resultant solution was lyophilized to dryness, taken up in 0.1 ml of 12 M perchloric acid and digested at 100°C for 2 hours. (This treatment removes the sugar moiety from the pyrimidine base (9). The controls, described in the figure, were subjected to the same treatment, omitting radiation where indicated. After neutralization with 4 N NaOH, 50 μl, of the hydrosylates from the various samples were spotted in different lanes of the same piece of Whatman #1-3 MM paper and subjected to paper electrophoresis at 3000 volts and pH = 3.6 for forty minutes. The resultant paper was divided crosswise into equal width strips large enough to contain ninhydrin positive spots located in earlier experiments and lengthwise into the channels corresponding to each originally spotted sample. The resultant strips were assayed for tritium activity using scintillation counting. The cpm plotted along the ordinate, for strips other than the photolyzed thymidine-lysine system, are normalized values. These normalized values were obtained as follows. The number of counts in each strip in a particular lane was first multiplied by a factor obtained by dividing the total cpm spotted in the photolyzed thymidine-lysine lane by the number of cpm applied to that lane; this data was obtained by counting 50 μl of each of the hydrosylates to be applied. The resulting number of cpm in each lane was then multiplied by a factor which made the number of cpm in the strip containing thymine (strip A) the same as that contained in strip A in the photolyzed thymidine-lysine system (8648 cpm). Strips B and E contained strongly ninhydrin positive material for the photolyzed thymidine-lysine system; the corresponding strips for the controls did not show reactivity

were not photolyzed or did not contain lysine, showed that photolysis was required for appearance of the products contained in strips B and E and that the presence of lysine was required as well. It should be emphasized that the products contained in strips B and E are those which survived acid hydrolysis. Electrophoresis of irradiated thymidine-lysine systems (or lysine-thymine systems) without hydrolysis gave several additional ninhydrin positive spots containing radioactivity.

Figure 2 presents the results found when DNA, containing tritium labeled thymine, is irradiated, hydrolyzed, and subjected to paper electrophoresis. There is a relatively large amount of radioactivity associated with strip B for the irradiated DNA-lysine systems. The controls show much smaller amounts of radioactivity contained in strip B. Further, the material contained in strip B is ninhydrin positive, indicating that lysine is incorporated into the compound(s) containing the tritium label. As thymine is the only base containing the label, and since the acid treatment used is sufficient to completely hydrolyze the DNA to free bases, the material in strip B must contain thymine-lysine adduct (or adducts). The fact that the product contained in strip B for the DNA-lysine system moves at the same rate under paper electrophoresis as the product contained in strip B in the thymidine-lysine system suggests that they are the same.

There is no substantial evidence that the product obtained in strip E in the thymidine-lysine system is also formed in the DNA-lysine system. Perhaps steric constraints, limiting accessibility of the lysine side chain to particular reactive sites within the photo-excited thymine, prevents the second product from being readily formed in the DNA-lysine system.

We are currently working to determine the structure of the thymine-lysine adducts to determine the dose dependence of adduct formation in lysine-DNA

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toward ninhydrin. There is a strongly ninhydrin positive spot beyond strip F in systems #2 and #4 corresponding to lysine; no radioactivity was found in this area.

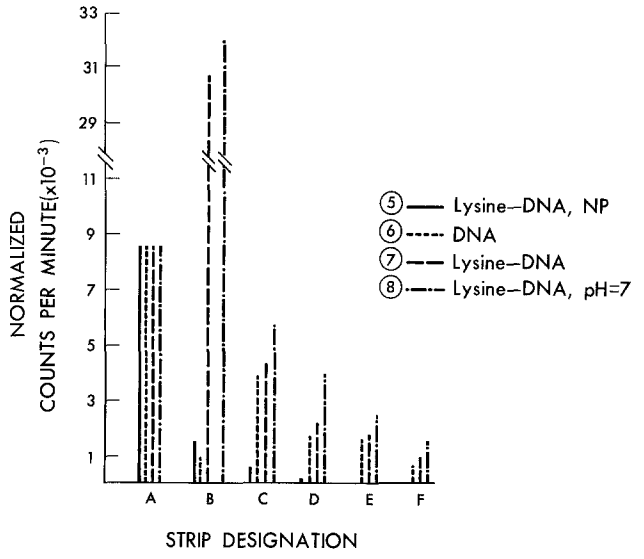

Figure 2

The photochemical addition of lysine to DNA. A typical protocol is as follows. A 0.2 ml sample of aqueous solution containing 1.52 mg of lysine (0.05 mole/liter) and 0.01-0.02 mg of DNA (HeLa) containing thymine, tritium labeled on the 5-methyl (specific activity of 1 x 10^6 dpm/μg), was placed in a quartz NMR tube. The irradiation, as described in Figure 1, was for 36 hours. The work up and electrophoresis was as described in Figure 1. Normalization was similar to that carried out for the thymidine-lysine system and was relative to the number of counts contained in strip A of the photolyzed thymidine-lysine system. Correspondence of fractions A, B . . . in the DNA-lysine system to those in the thymidine-lysine system was established by subjecting samples from both sets in electrophoresis on the same sheet of paper. In addition to the activity shown in the figure, some radioactivity remained close to the origin in a strip preceding A. System #8 was buffered at pH = 7 during photolysis by use of phosphate buffer. The labeled DNA used in the experiment was obtained from HeLa cells grown for 2 days in 0.5 mCi/ml of thymidine tritiated on the 5-methyl group. Nuclei were prepared as described in (10). The DNA was then prepared by deproteinization in 1% SDS in the presence of 1M $NaClO_4$ (11).

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systems, and to establish whether thymine-lysine adducts are also formed in irradiated DNA-histone systems and in irradiated chromatin.

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