α-S-CYSTEINYL-5,6-DIHYDROTHYMINE: A POSSIBLE MODEL FOR RADIATION-INDUCED CROSS-LINKING OF DNA AND PROTEIN

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Summary

α-S-cysteinyl-5,6-dihydrothymine is identified as one of the major products of thymine irradiated in the presence of L-cysteine hydrochloride with ultraviolet light (λ>230 nm).

In a series of papers, Smith (1,2,3) has presented evidence to indicate that addition of amino acids to pyrimidine bases is one possible mechanism for radiation-induced cross-linking of nucleic acids to proteins. Smith has corroborated his initial findings by the identification of 5,S -cysteinyl-5,6-dihydropyrimidines (I) as photoaddition products of pyrimidines with cysteine (4,5). In our attempts to study the kinetics and mechanisms of the photochemical addition of cysteine to pyrimidine bases, we observed that the behaviour of thymine was significatnly different from that of uracil when irradiated in the presence of cysteine. Subsequent investigations have resulted in the identification of a new product, α-S-cysteinyl-5,6-dihydrothymine (II), as one of the major photoaddition products of thymine to cysteine. The properties of this unusual product, in which the cysteine residue is attached to the methyl group of thymine, are described in this communication.

Methods

The solution for the irradiation was prepared by dissolving 3 mmoles of thymine, 12 mmoles of cysteine hydrochloride and 20 μc of thymine-2-C^{14} in 750 ml of water. A water-cooled high pressure mercury lamp enclosed in a vicor jacket was placed in the solution which was kept cold by running water through an outer jacket. A stream of nitrogen was bubbled through the solution for one hour prior to irradiation and the bubbling was

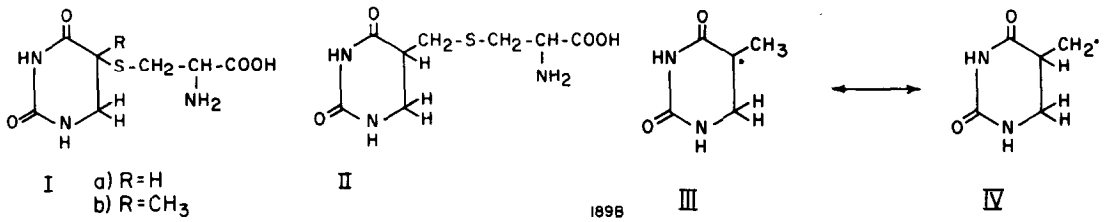
continued during the irradiation (6 hours). The irradiated solution was concentrated to about 25 ml and filtered. The filtrate was applied on a DOWEX 50W X12 CH^+ .100-200 mesh) column (34 x 2cm). By washing the column with water until the washings had neither radioactivity nor ultraviolet absorbance above 220 nm, the unreacted thymine was recovered. Trace amounts of cyclobutane thymine dimer, and dihydrothymine, were also present in the water eluate. Subsequently the column was eluted with a hydrochloric acid gradient (50 ml of con. HCl and 450 ml of water in the mixing chamber; 100 ml of conc. HCl and 400 ml of water in the reservoir). 20-ml fractions were collected and aliquots from each fraction were counted for radioactivity. Two radioactive peaks were detected. Paper chromatographic analysis of the second peak (Fractions 13-18) showed the presence of a number of compounds, including Ib. Other products in this second peak will be discussed elsewhere.

Paper chromatography (n-butanol: acetic acid: water; 80:12:30) of the first peak (Fractions 6-11) revealed the presence of only one radioactive component (R_F 0.10). From the chromatogram, the radioactive region was cut out, extracted with 0.1 N HCl, the extract was evaporated to dryness and the residue was crystallized from alcohol: water (1:1). We will show this product to be II.

Results and Discussion

The radioactive region on the paper chromatogram developed a purple colour with ninhydrin and a yellow colour with Ehrlich's reagent after pretreatment with alkali (6). Treatment of the product with Raney Nickel yielded dihydrothymine and alanine (5). An aqueous solution of the product did not have a maximum in the 240-280 nm region. The infrared absorption spectrum (KBr pellets) of the product has bands (cm^{-1}) at 1745 (carbonyl), 1730 (amino acid COOH), 1695 (ureido carbonyl) 1640, 1630, 1495 ($-NH_3^+$) and 1245.

These results indicate that the radioactive component is a dihydrothymine derivative containing an α-amino acid group and the CH_2-CH group is attached to the thymine moiety through sulphur.



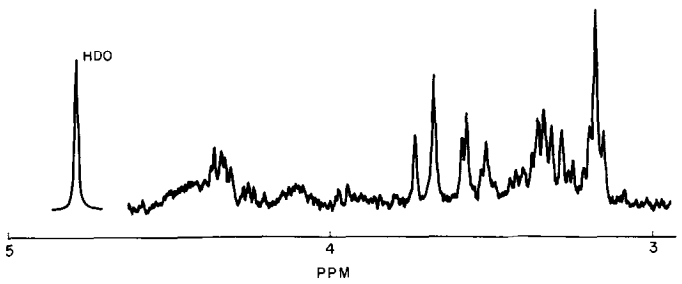


Figure 1. 220 MHz Pmr spectrum of II in D_2O, using TMS external standard (taken at the 220 MHz NMR center. Ontario Research Foundation, Sheridan Park, Ontario, Canada).

The product was stable in acid solutions. After heating at 100° for 15 min in 6 N HCl more than 80% of the product could be recovered. In alkaline solutions (pH 12) at 23° it underwent ring-opening with a half life of about 6 minutes.

The proton magnetic resonance spectrum (Fig. 1) of the product was consistent with structure II. The absence of a signal for the methyl group and signals for three $-CH_2-$ groups provide direct evidence that the methyl group of thymine is involved in the addition. The methine proton of the amino acid appears as a quartet at δ4.28. The 6-methylene protons give rise to an AB quartet with doublets centred at δ3.7 and δ3.56 with J_{AB} = 14 Hz. The 5-methine proton gives rise to the multiplet at δ3.42. The multiplet at δ3.32 can be attributed to the α-methylene protons. The methylene protons of the amino acid group appear at δ3.18. The vicinal coupling of the C_5 proton to C_6 protons of the order of 2 Hz suggests that the C_5 proton is in the equatorial position (7).

TABLE I. MASS SPECTRAL DATA

m/e	Relative Inetnsity	m/e	Relative Intensity
34	23	88	8
44	45	125	8
55	35	126	100
60	14	127	26
69	5	160	7
74	11	184	2
83	36	186	4

The very low volatility of the product resulted in a mass spectrum lacking a molecular ion. However, the fragmentation pattern (Table 1) provided valuable information to confirm the proposed structure. The peaks at m/e 34 (H_2S), 44 (CO_2) and at m/e 88 (CH_2 $CHNH_2$ COOH) are characteristic fragments of the cysteine residue (8). The intense peaks at m/e 55 and 83 are characteristic fragmentation ions of thymine (9). α-cleavage with rearrangement is probably responsible for the intense peak at m/e 126. Formation of an olefinic fragment carrying the positive charge is known to be a common fragmentation pathway for sulphides (10). The peak at m/e 160 is probably due to the fragment C_5 H_8 N_2 O_2 S^+.

Electron spin resonance (ESR) spectra studies have shown that UV-irradiation of thymine produces radicals (11). One of the major thymine radicals has been identified as III, which may also exist as IV. Annealing of IV by the addition of a thiyl radical is the most probable mechanism for the formation of II. Failure to identify IV by ESR studies is probably due to the low yield.

Addition across the C_5-C_6 double bond of thymine is generally considered as the major photochemical alteration of thymine. However, the identification of II as a photoaddition product of thymine suggests that alterations in the methyl group may also be significant under certain conditions. It is to be noted that the major thymine-derived photoproduct of bacterial spores, dry DNA and frozen DNA is 5-thyminyl-5,6-dihydrothymine, the formation of which involves

alterations in the methyl group of thymine (12,13). Since DNA-protein cross-linking is a major lesion in bacteria irradiated in the frozen state with ultraviolet light (14), photochemical addition of amino acid residues to the methyl group of thymine may play a role in UV-induced DNA-protein crosslinks.

A full paper describing three other new cysteine addition products and related compounds will be published shortly.

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