α-S-Cysteinylthymine: A Model for Protein–Nucleic Acid Cross-Linking†

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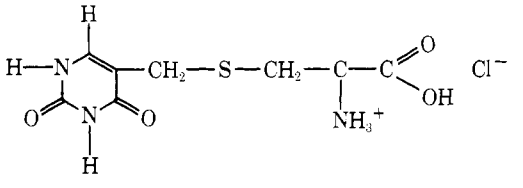
ABSTRACT: Crystals of α-S-cysteinylthymine, $C_8H_{12}ClN_3O_4S$, formula weight 281.72, are orthorhombic, space group $P2_12_12_1$, with a = 9.499 (1), b = 24.072 (4), and c = 5.012 (1) Å, V = 1146.1 (2) Å^3, and Z = 4. The structure was determined by the direct method and refined by a full-matrix least-squares procedure to a final residual, R = 0.043, using 1277 diffractometer data. From the structure, a three-dimensional model for the radiation-induced interaction of thymine residues and cysteine residues could be postulated.

The cross-linking of nucleic acids to protein is one of the lesions produced in biological systems by ultraviolet light (Smith and Hanawalt, 1969). This has been observed in bacteria (Smith, 1962; Alexander and Moroson, 1962; Bridges et al., 1967) and in mammalian cells (Alexander and Moroson, 1962; Habazin and Han, 1970; Han et al., 1975; Zimmerman et al., 1972). The radiation-induced binding of serum albumin to DNA (Braun and Merrick, 1975), DNA polymerase to DNA (Markovitz, 1972), and gene-5 protein to bacteriophage fd DNA (Anderson et al., 1975) provides direct proof at a molecular level for the cross-linking phenomenon. The role of DNA–protein cross-links in aging, carcinogenesis, and radiation biology has been recently reviewed (Smith, 1975).

Studies on model systems indicate that addition of pyrimidine bases to sulfhydryl amino acid residues is one mechanism for nucleic acid–protein cross-links (Smith and Aplin, 1966; Smith, 1970; Smith and Meun, 1968). Irradiation of thymine in the presence of cysteine (one model system) results in the formation of different products and one of them is α-S-cysteinylthymine (**1**) (Varghese, 1973). This product has also been isolated from calf thymus native DNA irradiated (λ ≃254nm) in aqueous solution in the presence of cysteine (A. J. Varghese, unpublished data). In addition, there is evidence that it is present in mammalian cells irradiated with ultraviolet light (Varghese and Rauth, 1974).

A detailed knowledge of the structure and conformation of radiation-induced amino acid adducts to DNA could yield important information about the relative orientation of these constituents inside a cell. With this as our aim, we have determined the structure of **1** from x-ray crystallographic data. The structure and a possible three-dimensional model for the radiation-induced interaction of cysteine with thymine in DNA are the subject of this paper.



Experimental Section

The crystal data are summarized in Table I. Three-dimensional data were collected on a Syntex $P\bar{1}$ automated diffractometer with monochromated Cu Kα radiation using the variable θ–2θ scan technique in the sin θ/λ range 0–0.603 Å^{-1}. Intensities were measured for 1402 reflections. The raw data were corrected for intensity loss by means of a linear plot derived from the change in intensity of measured standard reflections as a function of exposed time. Values of $\sigma(F)$ were derived from counting statistics and measured instrumental uncertainties. The data were reduced to a unique set of 1277 independent reflections (80% of the 1587 theoretically accessible) and converted to structure amplitudes. A Wilson plot of the data produced a scale factor to place the data on an absolute scale. No x-ray absorption correction was applied.

Results

Structure Determination and Refinement. The structure was solved by direct methods by means of the program MULTAN (Main et al., 1971). The first E map revealed all of the non-hydrogen atoms. After several cycles of isotropic and anisotropic least-squares refinement, a difference map was calculated which revealed the positions of the hydrogen atoms. Further refinement with anisotropic temperature factors (isotropic temperature factors for hydrogen) converged at residual, R, of 0.043, wR = 0.053. The weighting scheme was derived from an analysis of variance. For $F <$ 80, σ = 4.5; for $F > 80$, $\sigma = 4.5 + 0.35(F - 80)$.

The atomic scattering factors used for oxygen, nitrogen, carbon, sulfur, and chlorine were those in the International Tables for X-ray Crystallography (1962), and for hydrogen atoms those of Stewart et al. (1965). Figure 1 shows the molecule with the van der Waals atomic radii stippled in. Figure 2 shows the molecular conformation and the distances and angles in α-S-cysteinylthymine. Table II gives the atomic positional and thermal parameters. The observed and calculated structure factors are available on microfilm.

Description of the Structure. The thymine ring is planar and the bond angles and distances do not deviate significantly from those found in other thymine structures (Voet

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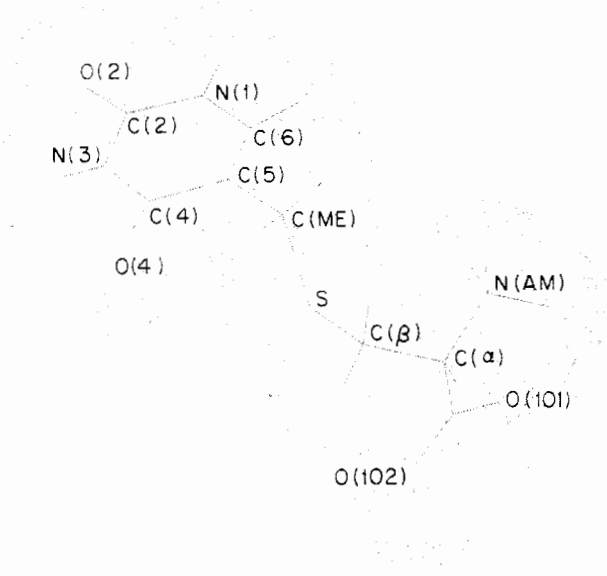

FIGURE 1: The molecule of α-cysteinylthymine showing the van der Waals radii.

Table I: α-*S*-Cysteinylthymine: Crystal Data.

Clear, colorless prisms
Orthorhombic, space group $P2_12_12_1$ (No. 19)
a = 9.499 Å
b = 24.072 Å
c = 5.012 Å
V = 1146.1 (2) Å^3
Z = 4
$F(000)$ = 584
λ = 1.5418 Å
d_x = 1.633 g cm^{-3}

similar. Here, the S atom is staggered and gauche with respect to the amino nitrogen atom and the carboxyl carbon atom. In the exceptional case the sulfur atom is also staggered but trans to the nitrogen atom. In each of these structures the nitrogen atom and one carboxyl oxygen atom are eclipsed; this seems to be generally true in amino acids.

A Model for One Kind of Addition of Cysteine to Thymine. Of importance is the relative position of the sulfur atom with respect to the thymine ring. Table III shows that

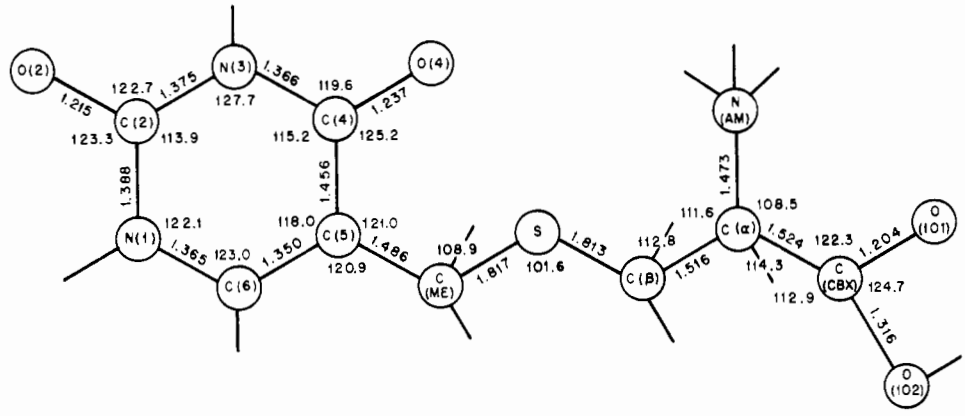

FIGURE 2: The molecule with distances and angles indicated. The average esd's of the bond lengths are 0.005 Å for bonds not involving H, 0.06 Å for bonds involving H, and of the bond angles, 0.5° for angles not involving H.

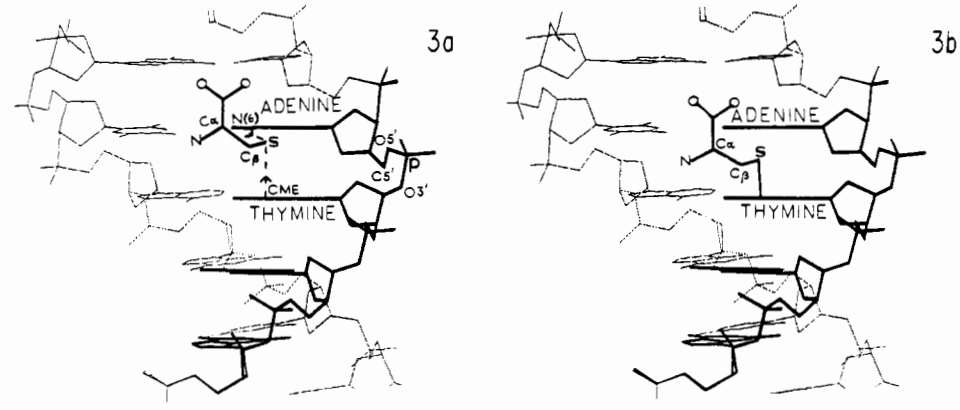

FIGURE 3: Models for the interaction of cysteine with thymine in DNA: (a) prior to irradiation; (b) after irradiation.

and Rich, 1970). The bond distances and angles in the cysteine moiety are in the range of expected values for a protonated amino acid.

The torsion angles of the cysteine portion given in Table III demonstrate that its conformation is similar to that observed in most other cysteine structures. In four out of five of these structures the conformation about C_α–C_β is very

the C(me)–S bond is approximately perpendicular to the plane of the thymine ring. The only other reasonable conformation corresponds to the torsion angle C(6)–C(5)–C(me)–S equal to +90°. Inspection of a model of DNA shows that this latter conformation is one which will allow the cysteine to join the thymine without being in close contact with the ribose-sugar backbone of DNA. One can pos-

Table II: Final Atomic Parameters.[a]

No.	Atom	X	Y	Z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
1	S	0.2981 (1)	−0.4258 (0)	0.1133 (2)	0.00291 (9)	0.00107 (2)	0.02196 (41)	0.00048 (3)	−0.00202 (20)	−0.00115 (7)
2	Cl	0.5867	−0.3382	−0.2325	0.00546	0.00116	0.02577	−0.00025	−0.00065	0.00032
3	O2	−0.1252 (4)	−0.2636 (2)	−0.4782 (8)	0.0067 (4)	0.0017 (1)	0.0345 (16)	0.0008 (1)	−0.0015 (7)	0.0041 (3)
4	O4	−0.0557	−0.3834	0.2045	0.0038	0.0013	0.0336	0.0002	0.0025	0.0037
5	O1O1	0.6496	−0.4963	−0.0944	0.0037	0.0017	0.0147	0.0006	0.0018	−0.0006
6	O1O2	0.4832	−0.5490	0.0928	0.0086	0.0013	0.0334	−0.0006	0.0061	−0.0021
7	N1	0.0982	−0.2706	−0.3086	0.0052	0.0009	0.0215	−0.0003	0.0017	0.0013
8	N3	−0.0847	−0.3226	−0.1323	0.0030	0.0012	0.0298	0.0002	0.0000	0.0002
9	N(am)	0.6305	−0.4156	0.2691	0.0026	0.0012	0.0185	−0.0001	−0.0007	−0.0007
10	C2	−0.0440	−0.2837	−0.3178	0.0048	0.0009	0.0261	0.0002	0.0010	0.0015
11	C4	−0.0025	−0.3487	0.0530	0.0033	0.0009	0.0242	0.0006	0.0025	0.0013
12	C5	0.1451	−0.3324	0.0528	0.0034	0.0008	0.0250	0.0002	−0.0009	−0.0001
13	C6	0.1874	−0.2943	−0.1278	0.0045	0.0008	0.0264	0.0001	0.0002	0.0000
14	C(me)	0.2470	−0.3581	0.2409	0.0054	0.0009	0.0273	0.0005	−0.0031	−0.0006
15	Cβ	0.3975	−0.4537	0.3915	0.0029	0.0010	0.0148	0.0003	0.0023	0.0001
16	Cα	0.5490	−0.4666	0.3196	0.0033	0.0009	0.0099	0.0005	0.0006	−0.0002
17	C(cbx)	0.5659	−0.5052	0.0808	0.0028	0.0010	0.0160	0.0009	−0.0018	−0.0003
18	HN1	0.130 (6)	−0.247 (3)	−0.395 (13)	3.3 (1.5)					
19	HN3	−0.185	−0.333	−0.160	3.9					
20	HN(am)1	0.627	−0.393	0.399	2.9					
21	HN(am)2	0.602	−0.397	0.129	2.8					
22	HN(am)3	0.748	−0.426	0.251	7.8					
23	HC6	0.278	−0.275	−0.137	8.2					
24	HC(me)	0.210	−0.368	0.417	2.8					
25	HC(me)1	0.328	−0.332	0.269	4.9					
26	HCβ	0.352	−0.492	0.437	2.0					
27	HCβ1	0.407	−0.426	0.513	3.9					
28	HCα	0.577	−0.485	0.479	2.5					
29	HO1O2	0.500	−0.574	−0.057	3.0					

[a] Positional parameters are given as fractional cell coordinates. Anisotropic temperature factor parameters are expressed as: $\exp[-¼(h^2a^{*2}B_{11} + k^2b^{*2}B_{22} + l^2c^{*2}B_{33} + 2hka^*b^*B_{12} + 2hla^*c^*B_{13} + 2klb^*c^*B_{23})]$ and isotropic temperature factors as: $\exp(-B\sin^2\theta/\lambda^2)$ with B_{ij} and B values given in Å^2. The standard deviations for each parameter, determined from the inverted full matrix, are given in parentheses and apply to the last specified digits.

Table III: Planes and Torsion Angles.

Plane of Thymine Ring[a]			
Atom	Distance from Plane (Å)	Atom	Distance from Plane (Å)
O(2)	−0.008	N(3)	+0.011
O(4)	−0.009	C(4)	+0.002
N(1)	−0.005	C(5)	+0.003
C(2)	+0.008	C(6)	−0.001

Torsion Angles in Cysteine Moiety	Value of Angle (°)				
Atoms defining Torsion Angle	α-*S*-Cysteinyl-thymine	Cystine[b]	Cysteine Orthorhombic[c]	Cysteine Monoclinic[d] I	Cysteine Monoclinic[d] II
C(6)–C(5)–C(me)–S	−99.0 (−sc)				
C(4)–C(5)–C(me)–S	79.3 (+sc)				
C(5)–C(me)–S–C(β)	−171.4 (−ap)				
C(me)–S–C(β)–C(α)	−119.4				
S–C(β)–C(α)–C(cbx)	−54.9	−53.4	−59.4	−50.6	68.7
S–C(β)–C(α)–N(am)	68.8	68.7	64.7	72.6	−170.1
C(β)–C(α)–C(cbx)–O_{101}	134.8	133.9	107.0	121.1	84.3
C(β)–C(α)–C(cbx)–O_{102}	−46.9	−46.9	−72.5	−59.4	−95.3
N(am)–C(α)–C(cbx)–O_{101}	9.6	9.5	−17.0	−3.0	−36.1
N(am)–C(α)–C(cbx)–O_{102}	−172.2	−171.2	163.5	176.4	144.4

[a] Equation of plane $-0.203x + 0.729y + 0.653z + 5.94 = 0$, where x, y, and z are the coordinates relative to the orthogonal axes. [b] Jones et al. (1974). [c] Kerr et al. (1975). [d] Harding and Long (1968).

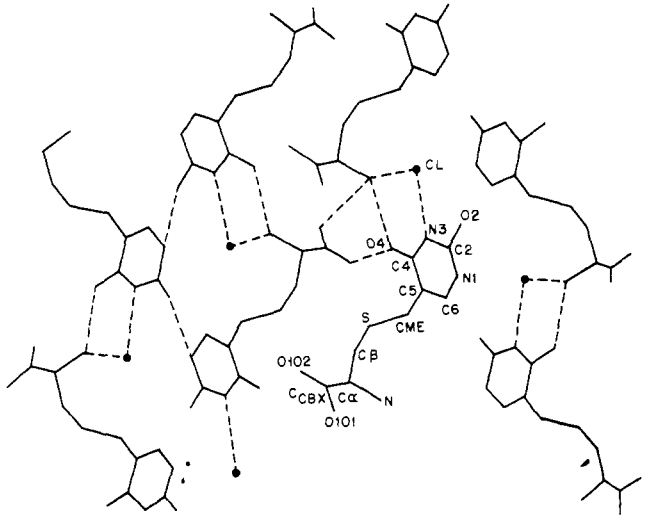

FIGURE 4: The hydrogen bonding in a crystal of α-cysteinylthymine chloride.

tulate from this observation one possible three-dimensional model for the interaction of cysteine with thymine by consideration of the four thymidine dinucleotides found in DNA: thymidine 3′,5′-deoxycytosine (dTpC), thymidine 3′,5′-deoxyguanosine (dTpG), thymidine 3′,5′-deoxyadenosine (dTpA), and thymidine 3′,5′-thymidine (dTpT). The sulfur atom of the cysteine can accept a hydrogen bond from the N(6) of the adenine in dTpA forming an N(6)–H···S hydrogen bond or the N(4) of the cytosine of dTpC forming a N(4)–H···S hydrogen bond. Typically an N–H···S hydrogen bond is 3.5 Å (Gabe et al., 1969; Rosenfield and Parthasarathy, 1975). At the same time the methyl can donate a hydrogen to the sulfur atom forming a C–H···S hydrogen bond of about 3.5 Å (Taylor et al., 1974). Then, only a small translation is necessary for the sulfur to bond covalently to the CH_2· radical produced by irradiation of thymine (Figure 3). The O(4) of the thymine in dTpT or O(6) of the guanine in dTpG cannot be hydrogen bond donors. They may accept a hydrogen from the sulfur and a C(me)···S interaction is not precluded.

Table IV: Hydrogen Bonding Distances.

Donor Atom (A)	Acceptor Atom (B)	Distance (Å)	Symmetry of B
O(102)	O(4)	2.629	$\frac{1}{2} - x, -1 - y, -\frac{1}{2} + z$
N(1)	O(2)	2.953	$\frac{1}{2} + x, -\frac{1}{2} - y, -1 - z$
N(3)	Cl	3.183	$-1 + x, y, z$
N(am)–H(Nam2)	Cl	3.157	x, y, z
N(am)–H(Nam1)	Cl	3.144	$x, y, 1 + z$
N(am)–H(Nam3)	O(4)	3.097	$1 + x, y, z$
N(am)–H(Nam3)	O(101)	3.054	$\frac{3}{2} - x, 1 - y, \frac{1}{2} + z$
C(6)	O(2)	3.002	$\frac{1}{2} + x, -\frac{1}{2} - y, -1 - z$

We thus propose a mode of hydrogen bonded interaction of cysteine with dinucleotide sequences (dTpC,dTpT,dTpG, and dTpA) in DNA as well as a possible explanation of its covalent attachment to thymine upon irradiation. It is important to note, however, that this particular model is likely when helical DNA and protein are irradiated. If the DNA which is irradiated is unwound, as for example during replication, the conformational constraints on both the cysteine residue and the nucleotide sequence would be lessened.

Hydrogen Bonding. All of the oxygen and nitrogen atoms participate in hydrogen bonding (Figure 4, Table IV) but the molecular packing in the crystal is dominated by the hydrogen bonds from three atoms to the chloride ion which also is in close contact with several other atoms.

Acknowledgment

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Supplementary Material Available

Structure factor data (5 pages). Ordering information is given on any current masthead page.

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