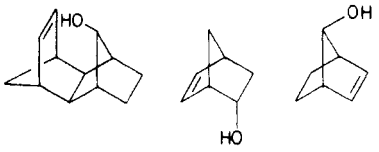
Chart II



C-H distance:	1.8 Å	2.2 Å	2.5 Å
EM:	2.3×10^4 M	35 M	0.85 M

lecularity problem is solvable.

Enzymes

Consider chymotrypsin, an enzyme which hydrolyzes amides with a 10^8 acceleration.[50] Now if there is one perplexing feature of this enzyme, it is the strikingly *dull* structure of its active site. The only catalytic groups present are a poor nucleophile (the serine hydroxyl) and a notoriously weak general base (the imidazole ring).[51] Why does this unimposing duo ravage amides so effectively? The answer seems simple if the behavior of the organic systems mentioned in the previous section is any guide: Chymotrypsin holds its catalytic groups and the amide carbonyl at bonding distances; this, plus a small general-base catalysis, more than suffices to explain the 10^8 acceleration. Work of Gerig and Reinheimer[52] agrees with this picture; they found that chymotrypsin binds cinnamic acid tightly enough so that "it does not have any freedom of motion independent of the motion of the enzyme". The construct is also consistent with the $>10^8$ rate increase observed in Winstein's norbornenyl tosylate (**15**). The main difference between the source of reactivity in **15** and that at an active site lies in the nature of the forces constraining the functional groups prior to reception of vibrational energy. In one case, the forces are covalent; in the other, noncovalent.

Holding two functionalities at a bonding distance requires energy, the largest portion of which relates to the need for "extruding" solvent. From where does this energy come? Clearly, there is only one source: binding energy. Stated in another way, the association constant between the enzyme and substrate is in actuality *smaller* than it would be if functional group proximity were not enforced upon the system. But once the critical distances are secured, the ensuing rate step can be extremely fast. *This, above all, is the lesson that organic chemistry gives to enzymology.* Alternative enzyme mechanism (electrostatic stabilization,[53] rack effects,[54] transition-state stabilization,[55] etc.) are intriguing but unnecessary.

This work was supported by the National Institutes of Health and the Army Research Office. The author would also like to thank Dr. George Hammond for provocative discussions. It was he who wisely advised, "Do not argue the physical truth; argue only as to whose model is the better aid to thinking."

(50) Bender, M. L.; Kézdy, F. J.; Gunter, C. R. *J. Am. Chem. Soc.* **1964**, *86*, 3714.
(51) An aspartate carboxyl is also near the active site, but its role in catalysis is uncertain. The main purpose may be to preclude the rotational freedom of the imidazole ring.
(52) Gerig, J. T.; Reinheimer, J. D. *J. Am. Chem. Soc.* **1970**, *92*, 3146.
(53) Warshel, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5250.
(54) Reference 2, p 282.
(55) Pauling, L. *Nature (London)* **1948**, *161*, 707.

Chemical Aspects of UV-Induced Cross-Linking of Proteins to Nucleic Acids. Photoreactions with Lysine and Tryptophan

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UV irradiation is known to have profound effects on a number of cellular functions. Until recently, photobiology has been primarily concerned with the photochemistry of pure DNA. However, as DNA does not exist in a cell in pure solution but in intimate contact with proteins and other biomolecules, it is conceivable that DNA–protein cross-links induced by UV light are important contributors to the deleterious effects of UV light on cells. Since the reports of Smith[1] and Alexander and Moroson[2] in the early 1960s showing that UV light induces cross-linking of proteins to DNA in living systems, there has been a substantial amount of evidence indicating that DNA–protein cross-linking is a major cause of UV-induced damage in biological systems.[3] The importance of these cross-links in aging, carcinogenesis, and radiation biology has been reviewed.[4] The tendency of proteins and nucleic acids to form specific covalent adducts as a result of UV irradiation is also used as a valuable tool for probing structural aspects of native protein–nucleic acid com-

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(1) Smith, K. C. *Biochem. Biophys. Res. Commun.* **1962**, *8*, 157.
(2) Alexander, P.; Moroson, H. L. *Nature (London)* **1962**, *194*, 882.
(3) For reviews: (a) Shetlar, M. D. *Photochem. Photobiol. Rev.* **1980**, *5*, 105. (b) Kornhauser, A. *Photochem. Photobiol.* **1975**, *23*, 457. (c) Smith, K. C. *Photochem. Photobiol. Nucleic Acids* **1976**, *2*, 187.
(4) Smith, K. C. Ed. "Aging, Carcinogenesis and Radiation Biology: The Role of Nucleic Acid Addition Reactions"; Plenum Press, New York, 1976.

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plexes.[5] This approach utilizes photochemistry to "freeze" existing contact points in the complexes, thereby allowing the identification of the interacting sites.

A variety of proteins has thus far been demonstrated to be cross-linked to DNA by UV irradiation, including such important chromosomal proteins as histones and RNA polymerase.[3] Exploration of the chemistry occurring in the UV-induced protein–nucleic acid cross-links is of central importance in achieving an understanding of the molecular aspects of UV-induced lesions in the genetic material. From the chemical standpoint, recent investigations[3a,6] have covered photoreactions of the individual bases of DNA with amino acids commonly occurring in proteins and their model compounds. For example, alcohols, which can be regarded as models for threonine and serine side chains, have been shown to react photochemically with purine and pyrimidine bases.[7] Several products were characterized from the UV-irradiated thymine–cysteine system.[8] A survey study showed that thymine could react with cysteine, cystine, lysine, arginine, tyrosine, and tryptophan.[9] Other studies have dealt with the scope of the reactivity of nucleic acid bases with amino acids and peptides.[10]

Despite many investigations, knowledge of the actual chemical nature of the adducts formed in UV-irradiated nucleic acid–protein systems is scant. For nucleic acid–protein complexes, in only a few cases have the actual identification of the bases and amino acids involved in cross-linking been reported. Uracil–methionine and uracil–tyrosine adducts have been shown to cross-link ribosomal proteins to rRNA,[11] but the structures of the adducts have not been elucidated. It has been suggested that thymine–cysteine adducts such as 5-(*S*-cysteinylmethl)uracil may be implicated in cross-linking of certain nucleic acid–protein complexes.[12]

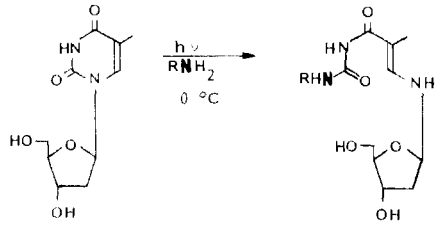
One major difficulty in the actual identification of nucleic acid–amino acid adducts from hydrolysates of irradiated DNA–protein systems is related to the very complex analytical problem, since small amounts of adducts are mixed into a very complex mixture of amino acids, nucleobases, and other photoproducts. Furthermore, it is not known whether the modified bases formed via cross-linking with amino acid residues are stable to the conditions required to convert nucleic acid–protein systems to their components. On the other hand, studies on closely related model systems would provide useful information that is directly relevant to achieving an understanding of the chemical aspects of the photo-cross-linking. However, another difficulty is associated with the relatively low reactivity of nucleic acid bases toward amino acids in general when these are irradiated together in solutions. The choice of proper model systems is primarily important in such studies.

Lysine is of particlar interest as a potential participant in protein–nucleic acid photo-cross-linking because of its occurrence in relatively large amounts in the histones, one of the main protein components of eukaryotic chromatin. The regions of the histones H2A, H2B, H3, and H4 that are most strongly involved in the binding of DNA to the core nucleosome in chromatin are generally believed to be those that are rich in lysine and arginine.[13] The potential proximity of these residues to the bases in DNA makes these amino acids of considerable interest as possible participants in UV-induced DNA–histone cross-links. Actually, it has been demonstrated that histones are cross-linked to DNA in chicken erythrocyte nuclei and chromatin by irradiation with 254-nm light.[14] We therefore initiated an extensive study on the photochemistry of the individual bases of DNA in the presence of basic amino acids such as lysine and arginine. In the course of this study we found an interesting photoexchange reaction of the N(1) nitrogen of thymine or thymidine with primary amines that proceeds efficiently upon irradiation with 254-nm with a number of compounds possessing primary amino groups. This type of photoexchange reaction has proved to be particularly important in photo-cross-linking of lysine residues of proteins to DNA. Furthermore, such photoexchange reactions have been shown to be effectively used for thymine selective modification of DNA, particularly for determining thymine residues in DNA sequencing.

Photochemistry of Thymidine in the Presence of Amines

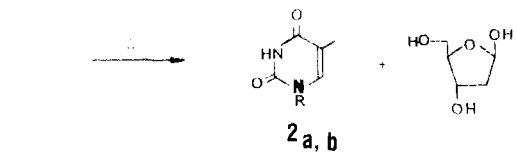
We first investigated the photochemistry of nucleic acid components in the presence of simple model compounds possessing primary amino and guanidino groups in order to know the chemical basis of UV-induced cross-linking involving lysine and arginine residues in DNA–protein systems. We found that upon UV irradiation primary alkylamines exhibit extraordinary high reactivity toward thymidine (Thd) among the four major deoxyribonucleosides contained in DNA.[15] Irradiation of an aqueous solution of Thd and *tert*-butylamine at 0 °C followed by preparative high-performance liquid chromatography (HPLC) gave rise to the formation of the ring-opened adduct **1a** (70%).[15b] The photoproduct **1a** was slowly converted to **2a** at ambient temperature. Irradiation at room temperature directly produced **2a** in high yield together with 2-deoxy-D-ribose. The overall process can be regarded as a "photoinduced exchange reaction" between Thd N(1) nitrogen and the alkylamine nitrogen (eq 1). A variety of amines including amino acids (vide infra) and polyamines undergo such photoexchange reaction with Thd. Irradiation of thymine (T) with *tert*-butylamine under

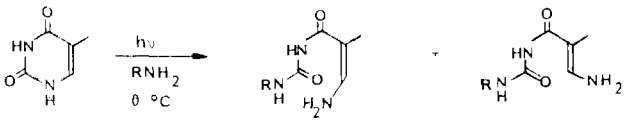
(5) (a) Sperling, J.; Harvon, A. *Biochemistry* **1976**, *15*, 1489. (b) Schimmel, P. R. *Acc. Chem. Res.* **1977**, *10*, 411.
(6) (a) Varghese, A. J., ref 4, p 207. (b) Saito, I.; Sugiyama, H.; Matsuura, T. *Photochem. Photobiol.* **1983**, *38*, 735.
(7) (a) Elad, D. *Photochem. Photobiol. Nucleic Acids* **1976**, *1*, 357. (b) Shetlar, M. D. *Photochem. Photobiol.* **1979**, *29*, 253.
(8) (a) Varghese, A. J. *Biochemistry* **1973**, *12*, 2725. (b) Fisher, G. L.; Varghese, A. J.; Johns, H. E. Photochem. *Photobiol.* **1974**, *20*, 109.
(9) Schott, H. N.; Shetlar, M. D. *Biochem. Biophys. Res. Commun.* **1974**, *59*, 1112.
(10) (a) Shetlar, M. D.; Christensen, J.; Hom, K. *Photochem. Photobiol.* **1984**, *39*, 125. (b) Shetlar, M. D.; Hom, K.; Carbone, J.; Moy, D.; Steady, E.; Watanabe, M. *Ibid.* **1984**, *39*, 138.
(11) (a) Zweib, C.; Brimacombe, R. *Nucleic Acids Res.* **1979**, *6*, 1775. (b) Maly, P. J.; Rinke, J.; Ulmer, E.; Zweib, C.; Brimacombe, R. *Biochemistry* **1980**, *19*, 4179.
(12) Paradiso, P. R.; Koningsberg, W. *J. Biol. Chem.* **1982**, *257*, 1462.
(13) Bradburg, E. M.; Maclean, N.; Matthews, H. R. Ed. "DNA, Chromatin and Chromosomes"; Blackwell Scientific Publications: London, 1981; p 21.
(14) Cao, T. M.; Sung, M. T. *Biochemistry* **1982**, *21*, 3419.
(15) (a) Saito, I.; Sugiyama, H.; Ito, S.; Furukawa, N.; Matsuura, T. *J. Am. Chem. Soc.* **1981**, *103*, 1598. (b) Saito, I.; Sugiyama, H.; Matsuura, T. *Ibid.* **1983**, *105*, 956. (c) Saito, I.; Sugiyama, H.; Furukawa, N.; Matsuura, T. *Tetrahedron Lett.* **1981**, *22*, 3265.



(1)

similar conditions produced a 5:1 *E*–*Z* mixture of the ring-opened adduct **3** (eq 2).[15b]

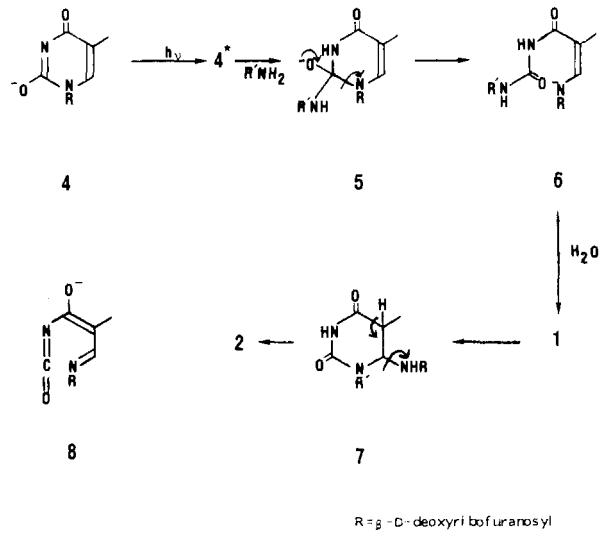




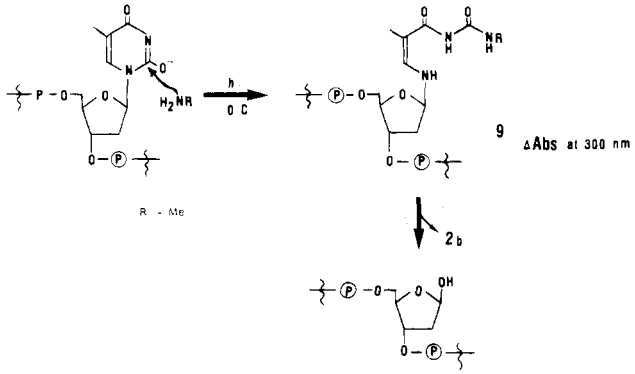
(2)

The quantum yield for the photoexchange reaction depends upon pH and amine concentrations. At acidic or neutral pH the photoreaction did not proceed, whereas the quantum yield increased with increasing pH in an alkaline pH region (pH 8–12). This result suggests the invervention of a photoexcited state of Thd monoanion **4** (pK_a = 9.8) in the adduct formation. In fact, N(3)-alkylated thymines such as 1,3-dimethylthymine did not undergo such photoaddition with alkylamines under basic conditions.[15c] Quantum yield of the photoreaction of Thd with methylamine (50 equiv) at pH 10.5 was 2.4×10^{-3}. Thus the photoreaction with methylamine in the alkaline pH region is an efficient process compared to other photochemical processes reported for Thd and T, e.g., photodimerization of Thd ($\phi = 5.6 \times 10^{-4}$)[16a] and T ($\phi = 4.7 \times 10^{-4}$)[16a] and photohydration of Thd ($\phi = 3 \times 10^{-6}$)[16b] in direct irradiation with 254-nm light at neutral pH. Acetone-sensitized irradiation of Thd in the presence of alkylamines never produced the ring-opened adduct but gave only a mixture of Thd photodimers,[15c] indicating that the lowest triplet state (π,π^*) of Thd monoanion **4** is not responsible for the adduct formation. Thus the most likely candidate for the excited species responsible for the photoexchange reaction is either the lowest singlet excited state or a vibrationally excited ground state of Thd monoanion **4**. The first step of the photoreaction is presumed to involve a nucleophilic attack of alkylamine on the C-2 position of photoexcited anionic species **4** to give **5**. Subsequent ring opening of **5** would yield the highly stabilized anion **6** by conjugation. Protonation of the anion **6** would give a *E*–*Z* mixture of the adduct **3** as was the case of T. Only the more stable *E* isomer **1** is isolable in the case of Thd. Upon being heated in aqueous solution, **1** would undergo intramolecular cyclization to **7** followed by β-elimination to yield **2**.[15b] An alternative mechanism involving isocyanate **8** formed by photochemical ring opening of **4** is unlikely since aqueous or alcoholic solvent should intercept **8**. This was not found to take place. This novel photoexchange reaction can be used for the synthesis of N(1)-substituted thymines of varied types and has proved to be useful for T-selective modification of DNA as described (Scheme I).

Scheme I



Scheme II

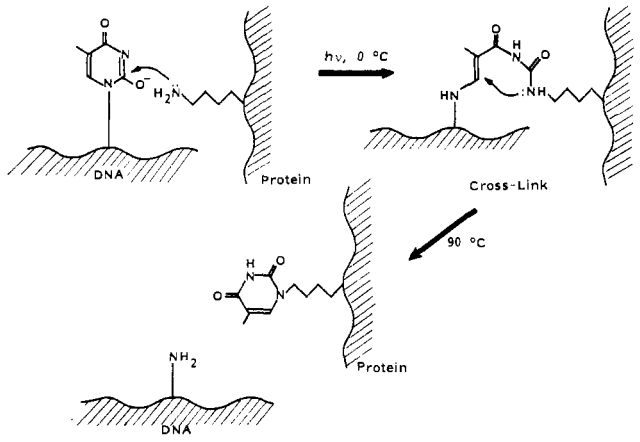


T-Selective Modification of DNA

When Thd and methylamine were irradiated at 0 °C in aqueous solution and the progress of the reaction was monitored with UV spectroscopy, a new absorption band of the ring-opened adduct **1b** appeared around 300 nm quantitatively.[15b] Moreover, high selectivity toward Thd has been observed when a mixture of four DNA components was irradiated in the presence of methylamine at 0 °C in aqueous solution containing a small amount of diazabicyclo[2.2.2]octane (Dabco), a singlet oxygen quencher, added to prevent guanosine photooxidation. Thus the photoreaction with methylamine under the specified conditions would be useful for T-specific modification of polynucleotides. Actually, we were able to demonstrate a convenient method for selective removal of T residues from DNA by utilizing this photoreaction as a key reaction.[15b] When heat-denatured calf thymus DNA in 0.1 M aqueous $NaHCO_3$ solution (pH 10.5) containing methylamine and Dabco was irradiated with 254 nm at 0 °C under the specified conditions, a new absorption band around 300 nm in-

(16) (a) Fisher, G. J.; Johns, H. E. *Photochem. Photobiol.* **1970**, *11*, 429. (b) Fisher, G. J.; Johns, H. E. *Ibid.* **1973**, *18*, 23.

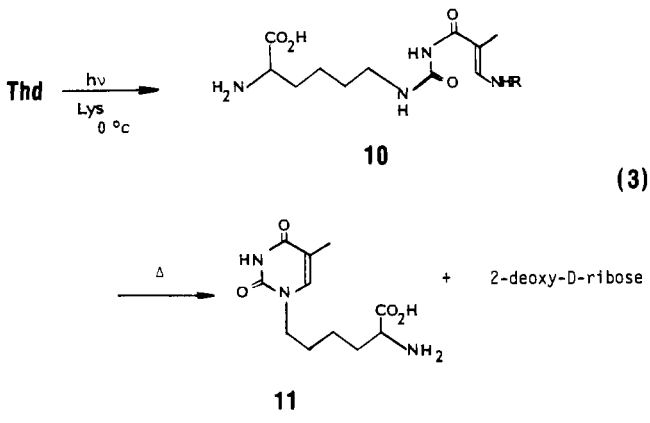
Scheme III



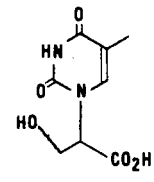
creased with increasing irradiation time with isosbestic points at 245 and 289 nm. The photolysate was then heated to 70 °C for 2 h. Upon this treatment the absorption at 300 nm mainly due to the ring-opened adduct **9** decreased with concomitant formation of 1-methylthymine (**2b**). The amount of reacted T residues of DNA is calculated from the absorbance change at 300 nm (ΔAbs) before and after heating the photolysate. 1-Methylthymine (**2b**) thus released from DNA was directly determined by HPLC without systematic degradations of DNA. The chemical processes occurring in irradiation of DNA in the preesence of methylamine are illustrated in Scheme II. It was also confirmed that irradiation of DNA fragments in the presence of methylamine followed by heating to 90 °C resulted in a strand scission at the reacting T residues (vide infra). The present method for T-selective modification is unique in that it allows the extent of DNA modification to be determined simply with UV spectroscopy or HPLC analysis without any tedious degradation of DNA.

Thymidine–Lysine Photoaddition

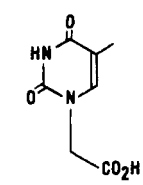
A similar photoaddition proceeds efficiently between Thd and ε-amino group of lysine. Irradiation of Thd and lysine with 254-nm light in distilled water (pH 10.5) at 0 °C followed by preparative HPLC gave the ring-opened adduct **10** in 70% isolated yield.[17] The adduct **10** was slowly converted to **11** in aqueous solution at room temperature with liberation of 2-deoxy-D-ribose (eq 3). Irradiation of Thd with other α-amino acids at the same pH did not produce the corresponding adducts. However, at more alkaline pH, e.g., pH >11, Thd reacts slowly with several α-amino acids such as serine, glycine, histidine, and asparagine to give the corresponding adducts **12**, **13**, **14**, and **15**, respectively, in moderate yields (20–45%).[18] At above pH 11 Thd or T react with lysine at both ε- and α-amino groups to give **11** and **16** with a ratio of ca. 4:1.[19] Recently, Shetlar and co-workers have reported that at strongly alkaline pH the uracil–lysine system undergoes a reaction similar to that of the Thd-lysine system but at a ca. 21 times slower rate.[19]



(Ser) (Gly)

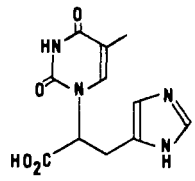


12

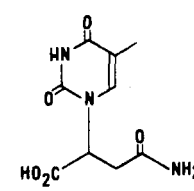


13

(His) (Asn)

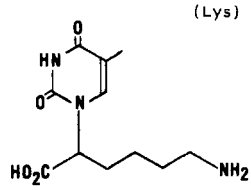


14



15

(Lys)



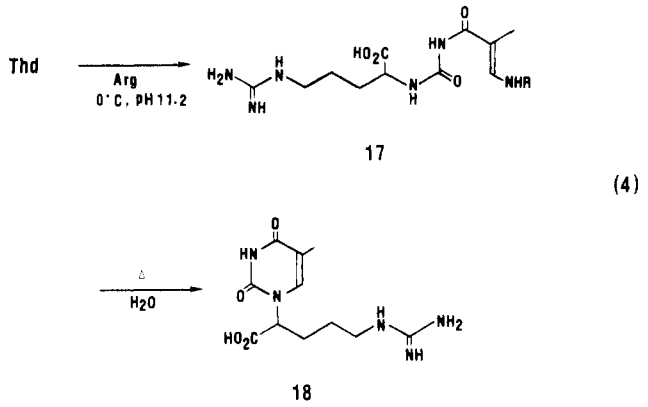
16

Irradiation of Thd and free arginine at pH 11.2 gave a different type of adduct **17** (30%).[17] Heating the aqueous solution of **17** gave **18** (eq 4). The result clearly indicates that the basic guanidino group of arginine cannot participate in the photoaddition with Thd. The exceptionally high reactivity of the lysine ε-amino group toward Thd at slightly alkaline pH suggests that the lysine ε-amino groups of proteins, intimately associated with T residues of DNA in DNA–protein complexes, may participate in photo-cross-linking. Cross-linking at the arginine sites appears to be of minor importance.

DNA–Histone Photo-Cross-Linking

The facile conversion of **10** to **11** under very mild conditions suggests that the cross-linked adduct formed by UV irradiation of DNA–protein complexes may re-

(17) Saito, I.; Sugiyama, H.; Matsuura, T. *J. Am. Chem. Soc.* **1983**, *105*, 6989.
(18) Saito, I.; Sugiyama, H.; Gupta, M. B.; Matsuura, T. 9th International Conference of Heterocyclic Chemistry, Tokyo, 1983; p 160.
(19) Shetlar, M. D.; Taylor, J. A.; Hom, K. *Photochem. Photobiol.* **1984**, *40*, 299.



lease a free protein containing modified lysine residues by a similar thermal reaction as illustrated in Scheme III. In order to probe this possibility, we examined the UV irradiation of nucleohistone. Irradiation of calf thymus nucleohistone in $NaHCO_3$–Na_2CO_3 buffer at pH 10.5 was conducted at 10 °C with 254-nm. Following irradiation, the solution was heated to 70 °C, lyophilized to dryness, and then subjected to acid hydrolysis.

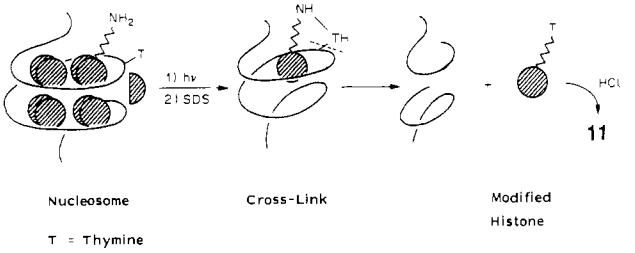
Analysis of the hydrolysate by HPLC or an amino acid analyzer revealed the presence of thymine–lysine adduct 11 which was isolated by HPLC and identified by ^1H NMR.[17] The yield of 11 increased proportionally with increasing irradiation time within 40 min, and further irradiation did not increase the yield significantly. This result may reflect the fact that the lysine ϵ-amino group in intimate association with T residues of DNA is primarily responsible for the formation of 11. Irradiation at neutral pH (pH 7.5) also produced the same adduct, albeit in lower yield, in contrast to the model experiment where a higher pH is necessary for the adduct formation.

We next examined the irradiation of chicken erythrocyte nuclei in the same buffer (pH 9.5). After being heated to 90 °C, the photolysate was hydrolyzed and then analyzed by an amino acid analyzer to again reveal the presence of 11 in the hydrolysate. None of the control experiments without irradiation or irradiation with Pyrex-filtered light (>280 nm) produced 11.[17] These experiments clearly demonstrated that irradiation of DNA–histone systems with 254-nm light followed by acid hydrolysis leads to the formation of 11. Thus we were able to confirm that the reaction sequence represented in Scheme III is actually occurring upon irradiation of DNA–histone systems. It could not be stated with certainty, however, that only the histones in their native nucleosomal state were responsible for the production of 11 because of the possibility of contamination by small amounts of other proteins in the nucleohistones used in our experiments.

There have been several reports on the formation of DNA–histone adducts upon UV irradiation of chromatin.[14,20] For example, Kunkel and Martinson[20b] have observed that a considerable amount of reversal of DNA–histone cross-links occurred in irradiation of calf thymus nuclei, i.e., the histones photo-cross-linked to DNA are released upon rechromatography on Sepharose column. We also observed that heating the crude DNA–histone adducts releases free histones which are

Scheme IV

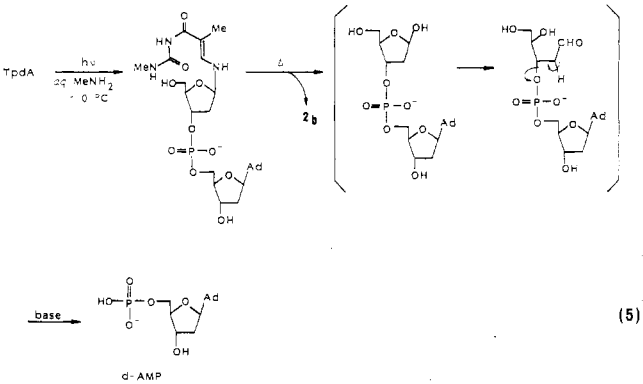
Schematic Representation of the Proposed Mechanism for Photo-Cross-Linking in Nucleosome



detectable by gel electrophoresis.[21] These adducts were separated on ultracentrifugation by adding $NaDodSO_4$ to UV-irradiated chicken erythrocyte nuclei in $NaHCO_3$–Na_2CO_3–EDTA buffer. Knowing the chemical nature of **10** and confirmation of the presence of 11 in the acid hydrolysate of the cross-linked adducts makes it highly probable that the apparent reversibility is due to the cross-links of the lysine ϵ-amino groups of histones to T residues of DNA. In the subsequent chemical reaction induced by heating the DNA–histone adducts, there occurs a release of free histones that contain partially modified lysine residues. It was confirmed that irradiation of DNA fragments with primary alkylamines and subsequent heating induce DNA strand scission at the sites of reactng T residues.[22] Therefore, the overall process initiated by UV irradiation of nucleosome may be schematically represented as in Scheme IV. Such a type of cross-linking may be relevant to the UV-induced damage to DNA in cells.

T-Specific Cleavage of DNA Fragments with Spermine

We observed that irradiation of a dinucleotide, i.e., thymidylyl-(3′–5′)-2′-deoxyadenosine (TpdA), in the presence of primary alkylamines resulted in the formation of a ring-opened adduct of the pyrimidine ring which on subsequent heating leads to the cleavage of the 3′–5′-phosphodiester linkage to produce 5′-AMP via β-elimination (eq 5).[15b] We also know from the model



experiments that the cleavage does not require piperidine treatment. We thought that the sequence of the reactions might be used for T-specific cleavage of DNA fragments. With information of these model experiments in hand, we devised a new, convenient method for highly T-selective cleavage of DNA fragments by utilizing a photoreaction with spermine which is known

(20) (a) Sperling, J.; Sperling, R. *Nucleic Acids Res.* **1978**, *5*, 2755. (b) Kunkel, G. R.; Martinson, H. G. *Ibid.* **1978**, *5*, 4263. (c) Mandel, R.; Kolomitjtseva, G.; Brahms, J. G. *Eur. J. Biochem.* **1976**, *211*, 1222. (d) Mee, L. K.; Adelstein, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2194.

(21) Saito, I.; Sugiyama, H.; Matsuura, T. *Nucleic Acids Res., Sp. Publ.* **1982**, No. *11*, 225.

(22) Saito, I.; Sugiyama, H.; Matsuura, T.; Ueda, K.; Komano, T. *Nucleic Acids Res.* **1984**, *12*, 2879.

to strongly bind to DNA and may act as an inhibitor for guanine photooxidation.[22]

Figure 1 illustrated the autoradiographs of the sequencing gels of UV-irradiated 3′-[^{32}P]-end-labeled DNA fragment (178 bp) together with the standard sequencing reactions (G, G + A, C + T, C) of the Maxam–Gilbert method. Irradiation for 10–30 min with a germicidal lamp emitting mainly 254-nm light in the presence of 1 M spermine resulted in a T-specific cleavage of the DNA chain (lanes a, b, and c). When the same irradiation was followed by treatment with 1 M piperidine (90 °C, 30 min), the strong T bands were accompanied with less intensive bands at G, cytosine (C), and adenine (A) residues (lane d). The G cleavage is probably due to the photooxidation of G residues, whereas the minor C cleavage is assumed to result from alkaline-labile lesions at pyrimidine–cytosine sequences recently observed by Haseltine and co-workers in the 254-nm irradiation of DNA.[23] When the DNA fragment was irradiated with methylamine, both G and T bands with intensity of G > T appeared without piperidine treatment (lane e), indicating that spermine retards the G reaction. A similar T-specific cleavage has also been observed in the irradiation of a 5′-[^{32}P]-end-labeled DNA fragment with spermine. In this case, however, prolonged heating (90 °C, 10 min) of the irradiated DNA fragment is necessary to attain a T-specific reaction.

With a single exposure to UV light for 20 min, we were able to indentify T residues in sequences up to 100 base pairs in length. While the Maxam–Gilbert method based on base-selective chemical reactions of end-labeled DNA has been widely used for sequencing DNA, the pyrimidine specific reactions using hydrazine are somewhat less reliable than those used for purines, and their specificity is sensitive to reaction conditions.[24] The present T-specific reaction with spermine and the G > T reaction with methylamine may be conveniently used in combination with the standard Maxam–Gilbert's C + T reaction to provide independent confirmatory readings. A significant advantage of our method is the elimination of the step involving the time-consuming piperidine treatment. The results also indicate that photoreaction of DNA with neighboring primary amino groups results in a strand scission as the sites of reacting T residues upon mild heating under slightly alkaline conditions.

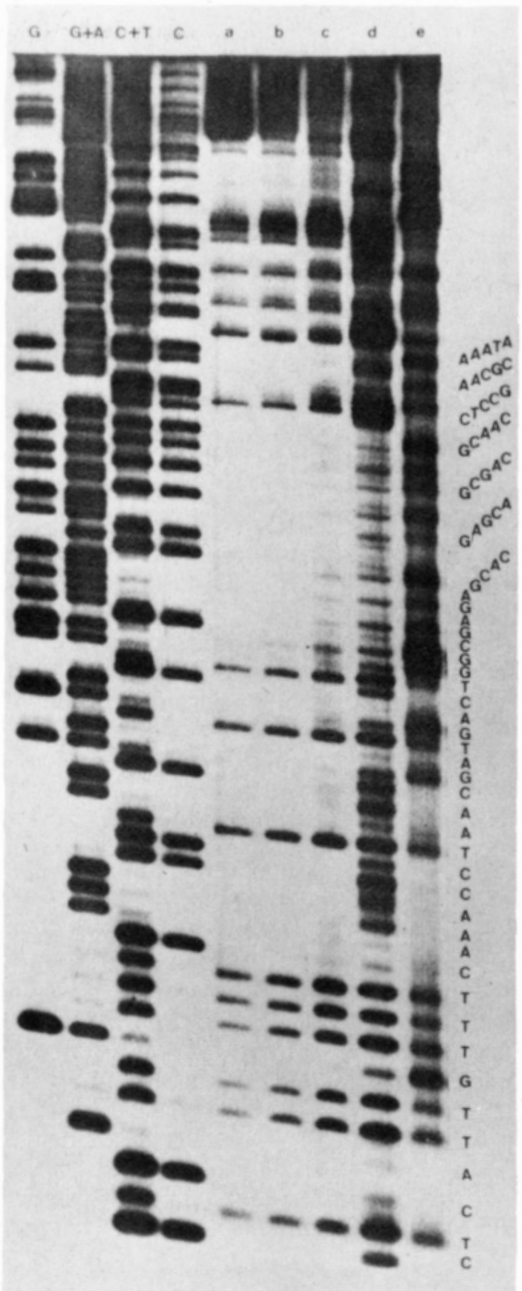


Figure 1. Photoreaction of a long DNA fragment (178 bp) in the presence of various amines. In lanes a, b, and c, the irradiation was performed in the presence of spermine for 10, 20, and 30 min, respectively. In lane d, 20-min irradiation with spermine was followed by treatment with 1 M piperidine (90 °C, 30 min). In lane e, the irradiation was performed for 20 min in the presence of methylamine.

Photocoupling of 5-Bromouridine to Tryptophan

The importance of the photo-cross-linking of DNA containing 5-bromouracil (BrU) to various proteins, e.g., histones, RNA polymerase, and *lac* repressor, has been recognized in photobiology.[3a] The replacement of T in DNA by BrU is also used for investigating the contact position located in macromolecular complexes as a bifunctional bridging agent.[25] However, very little has been known about the nature of the amino acid–nucleic acid adducts. Sulfhydryl compounds such as cysteine and glutathione were reported to undergo photocoupling to BrU.[26] As a model for such photo-cross-linking, photoreaction of BrU with a variety of indoles including tryptophan derivatives has been examined.[27] N^b-Acetyltryptophan ester (**19**) undergoes an efficient photocoupling to 2′,3′-*O*-isopropylidene-5-bromouridine (**20a**) or 5-bromo-1,3-dimethyluracil (**20b**) on acetone sensitization in acetonitrile to give **21** in a regiospecific fashion (eq 6). Involvement of triplet BrU (**20b**) in the photocoupling reaction was demonstrated by sensitization experiments. Solvent and substituent effects as

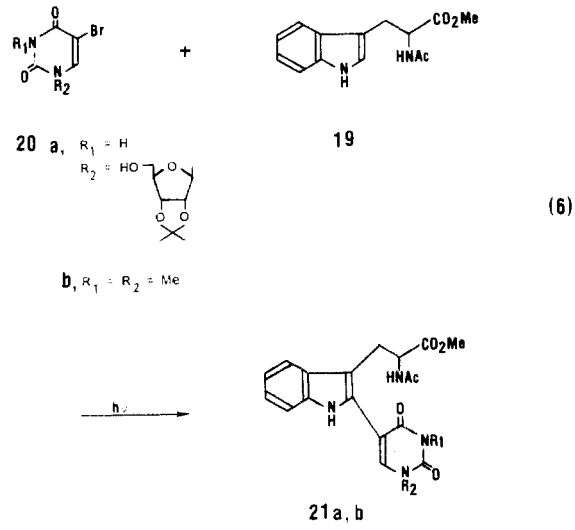
(23) Lippke, J. A.; Gordon, L. K.; Brach, D. E.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 3388.

(24) (a) Friedmann, T.; Brown, D. M. *Nucleic Acids Res.* **1978**, 5, 615. (b) Rubins, C. M.; Schmid, C. W. *Ibid.* **1982**, *8*, 4613. (c) Simoncsits, A.; Török, I. *Ibid.* **1982**, *10*, 7959.

(25) (a) Bahl, C. P.; Wu, K.; Itakura, K.; Katagiri, N.; Narang, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 91. (b) Goeddel, D. V.; Yansura, D. G.; Caruther, M. H. *Ibid.* **1978**, *75*, 3578.

(26) Varghese, A. J. *Photochem. Photobiol.* **1974**, *20*, 461.

(27) (a) Saito, I.; Ito, S.; Matsuura, T. *J. Am. Chem. Soc.* **1978**, *20*, 461. (b) Ito, S.; Saito, I.; Matsuura, T. *Ibid.* **1980**, *102*, 7535.



20 a, R_1 = H, R_2 = HO; b, R_1 = R_2 = Me

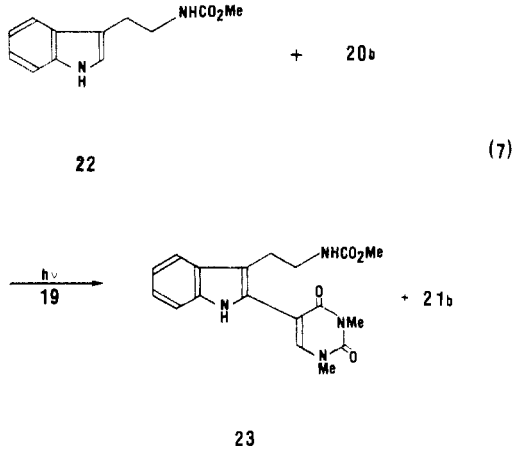
19

21a, b

(6)

well as the inhibitory effect of electron-transfer quenchers, e.g., *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine or 1,2,4,5-tetracyanobenzene, have suggested that the acetone-sensitized photocoupling of **19** to **20** proceeds via an electron-transfer process.[27b] Regiospecific coupling at the C-2 position of the indole ring also suggests an electron-transfer process from **19** to **20b** since the calculated highest positive charge of indole cation radical is on the C-2 position.[28] Thus the triplet exciplex formed from triplet **20b** (BrU) and **19** (InH) is assumed to dissociate to $InH^{+}\cdot$ and $BrU^{-}\cdot$ in a polar solvent like acetonitrile. The anion radical $BrU^{-}\cdot$ would release the Br anion yielding the 5-uracilyl radical which combines with $InH^{+}\cdot$ followed by deprotonation to produce the coupled product **21b** (In–U) (Scheme V).

Further support for the intervention of an electron-transfer process has been obtained in the photoreaction with N^{b}-(methoxycarbonyl)tryptamine (**22**). Acetone-sensitized irradiation of **22** with **20b** did not produce the coupled product. However, in the presence of **19** (E^{ox} = 0.82 V vs. SCE) as an electron carrier, **22** (E^{ox} = 0.75 V vs. SCE) reacted smoothly with **20b** to yield **23** at the expense of the coupled product between **19** and **20b** (eq 7).[27b] A similar double electron transfer



22

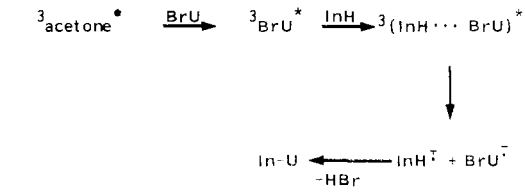
23

(7)

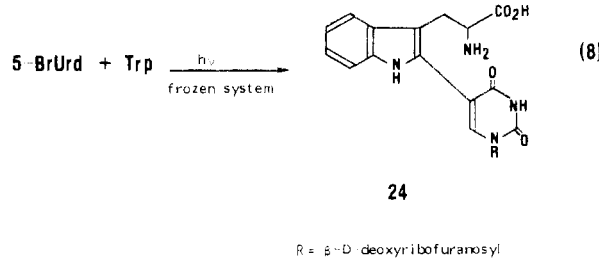
has also been observed in the photoreaction using 2-methoxynaphthalene as an electron carrier in place of **19**.[29] Recently, Koch and co-workers have proposed a one-electron-transfer mechanism for the reaction of triplet BrU with 2-propanol leading to debromination.[30]

(28) Yoshida, K. *J. Am. Chem. Soc.* **1979**, *101*, 2116.
(29) Ito, S.; Saito, I.; Matsuura, T. *Tetrahedron Lett.* **1979**, *20*, 4067.

Scheme V



In contrast to the acetone-sensitized photocoupling in organic solvents, irradiation of free tryptophan (Trp) and 5-bromouridine (BrUrd) in aqueous fluid solution failed to produce the coupling product. However, irradiation of an aqueous frozen solution of Trp and BrUrd gave the corresponding Trp–uridine adduct **24** (eq 8).[31] The following observations suggest that this



24

R = β-D-deoxyribofuranosyl

(8)

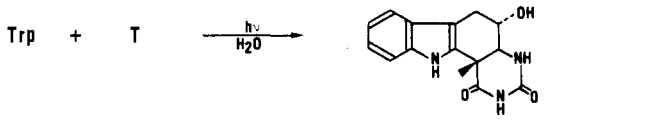
photocoupling may proceed via a mixed aggregate formation between Trp and BrUrd in frozen system. (i) The emission of Trp was efficiently quenched by only 0.025 equiv of BrUrd at 77 K, while the fluorescence emission at ambient temperature was not quenched appreciably by equimolar BrUrd in aqueous fluid solution. (ii) Addition of organic solvents or mineral salts such as acetone or NaCl to the frozen system completely inhibited the formation of **24** as a result of the prevention of aggregate formation by these additives. A similar photocoupling would be expected to occur between BrU-containing DNA and Trp residues in proteins. In such case the coupled product may be readily detectable by its characteristic fluorescence emission at 460 nm.[27b]

Photoreaction between Tryptophan and Thymine

In our studies on the photoreaction between DNA components and amino acids above-mentioned, light absorption by pyrimidine bases has been the primary event. However, there is a possibility that light absorption of aromatic amino acids such as tryptophan and tyrosine residues of proteins might induce chemical reactions in protein–nucleic acid assemblies. Reeve and Hopkins recently reported that irradiation (>260 nm) of Trp in the presence of T or uracil in aqueous solution at pH 7 produces two classes of photoproducts.[32] One was the dihydropyrimidine form of the bases and the other consisted of Trp–pyrimidine adducts, although the structures of the adducts have not been clarified. We were able to isolate and characterize two Trp–T photoadducts **25** and **26** by spectroscopic data and X-ray crystallographic analysis.[33] As shown in eq 9, both photoadducts are not the Trp–T adducts each

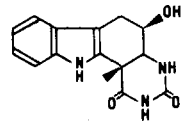
(30) Koch, T. H.; Swanson, B. J.; Kutzer, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 1274.
(31) Saito, I.; Ito, S.; Matsuura, T.; Hélène, C. *Photochem. Photobiol.* **1981**, *33*, 15.
(32) Reeve, A. E.; Hopkins, J. R. *Photochem. Photobiol.* **1979**, *30*, 677.
(33) Saito, I.; Sugiyama, H.; Matsuura, T.; Fukuyama, K.; Katsube, Y. *Tetrahedron Lett.* **1984**, *25*, 3243.

with an exact 1:1 stoichiometry as previously suggested by Reeve and Hopkins.[32] The Trp moiety suffers deamination and decarboxylation.



(9)

25



26

The formation of these photoadducts from Trp and T apparently involves a multistep photochemical and non-photochemical route. A similar type of adduct formation has been reported in irradiation of Trp–uracil[32] and Trp–poly U systems.[34] However, it is not known whether such adduct formation is responsible for the photo-cross-linking with Trp moieties of nucleoproteins.

(34) Reeve, A. E.; Hopkins, J. R. *Photochem. Photobiol.* **1980**, *31*, 413.

Concluding Remarks

In this Account, we have described a novel photoreaction between thymidine and alkylamines leading to ring-opened adducts. A similar photoreaction between thymine and lysine has proved to be particularly important in photo-cross-linking of lysine ϵ-amino groups of proteins to thymine residues of DNA in DNA–protein systems of biological significance, namely, the chromatin of eukaryotic cells. The present result is an example in which careful study on a chemical model system could directly contribute to the elucidation of the actual chemical processes occurring in irradiation of complicated molecular assemblies in biological systems. Achievement of a full understanding of the chemistry occurring in irradiation of DNA–amino acids, DNA–peptides, and more complex DNA–protein systems remains as a challenge to researchers in the fields of photochemistry, photobiology, and molecular biology.

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Excited-State Proton-Transfer Reactions and Proton-Induced Quenching of Aromatic Compounds†

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Proton association and dissociation in the excited states of aromatic compounds are elementary processes in both chemistry and biochemistry. The acid–base properties in the excited state of aromatic compounds are closely related to electronic structure, which is considerably different from that in the ground state. A large number of studies on the acidity constants pK_a^* in the excited state of aromatic compounds have been reported showing that the pK_a^* values are markedly different from the acidity constants in the ground state.[1-9] It is well-known that the pK_a^* values can be estimated by means of the Förster cycle,[1-3,10,11] the fluorescence titration curve,[2,3] and the triplet-triplet absorbance titration curve.[12] These methods involve the assumptions that proton transfer in the excited state is very fast and that acid–base equilibrium may

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† This paper is dedicated to Professor Ikuzo Tanaka (Tokyo Institute of Technology) on the occasion of his 60th birthday.

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