# Photoreactions of Thymine and Thymidine with *N*-Acetyltyrosine†

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ABSTRACT: We report here the photoinduced formation of a thymine–*N*-acetyltyrosine adduct. Irradiation of dilute solutions of thymine in the presence of *N*-acetyltyrosine (NAT) leads to the formation of *N*-acetyl-4-hydroxy-3-(6-hydrothymin-5-yl)phenylalanine (I), isolated as a mixture of the 5*R* and 5*S* diastereoisomers; the photoreaction occurs when irradiation is done either at $\lambda = 254$ nm or at wavelengths of $\lambda > 290$ nm. Irradiation of thymidine in the presence of NAT and of thymine in the presence of tyrosine leads to analogous photoadducts. The photoreaction of thymine with NAT is completely quenched by oxygen and cannot be sensitized by acetone. The likely mechanism involves initial photoionization of the amino acid and deprotonation to form the phenoxyl radical. Thymine then probably captures the released aqueous electron, leading to protonation at C6 of the resulting radical anion. Combination of the phenoxyl and 5,6-dihydrothymin-5-yl radicals would then lead to formation of the final products. The quantum yield for production of the thymine–NAT adduct at pH 7.8 was estimated to be about $5.5 \times 10^{-4}$, while a value of $2.3 \times 10^{-3}$ was estimated for production of corresponding thymidine adduct at pH 8.1. The dependence of the quantum yield for adduct formation on pH has been determined for both the thymine and thymidine reactions with NAT; the maxima in the quantum yield profiles occur at pH 8–8.5, while appreciable values were measured at pH 7.5. We have also demonstrated that a similar reaction occurs when tyrosine is located within a peptide. Angiotensin I, which contains a single tyrosine residue, was irradiated in the presence of thymidine and the resulting adduct isolated chromatographically. The position of attachment of the thymidine to the peptide was confirmed to be on tyrosine by tandem mass spectrometry, and the chemical nature of the adduct was verified by derivatization and cochromatography experiments.

Over the past three decades, a great deal of research has been devoted to understanding the macroscopic deleterious effects of ultraviolet (UV)[1] radiation on living systems in terms of photoreactions at the molecular level. Early research indicated that the action spectrum for cell killing closely follows the absorption spectrum of the nucleic acids, and it is now generally accepted that the mutagenic, carcinogenic, and lethal effects of solar radiation can, in great part, be accounted for by photoinduced modification of the genetic material to form nucleobase products and other chemical lesions (Wang, 1974). Although direct induction of effects by the action of UV radiation on nucleic acids implies absorption by the DNA ($\lambda < 300$ nm or so), the presence of exogenous and endogenous photosensitizers may give rise to important roles for the longer UVB and UVA wavelengths of the solar spectrum [for a review of UVB ($290 < \lambda < 320$ nm) and UVA ($\lambda > 320$ nm) induced DNA damage, see Peak and Peak (1989)].

The nature of photoinduced DNA modification has been extensively investigated. It has been found that photoinduced rearrangements of nucleobases and reactions between bases can occur, as well as reactions of the nucleobases with other cellular components having access to DNA (e.g., water and proteins). A number of photoproducts have been identified from studies with model systems and subsequently isolated from UV-irradiated DNA or DNA–protein systems, both in vitro and in vivo [for recent reviews, see Cadet and Vigny (1990) and Saito et al. (1990)]. The mechanisms of formation of these types of nucleobase products have, in a number of cases, been fairly well delineated (Cadet & Vigny, 1990) and, for some, the mechanisms by which cells repair such lesions have been categorized (Friedberg, 1985; Myles & Sancar, 1989). In recent years, a significant effort has been devoted to clarifying the nature of photoinduced DNA–protein cross-linking (Shetlar, 1980; Budowsky & Abdurashidova, 1989; Saito & Sugiyama, 1990). In our laboratory, we are particularly interested in determining the identities of the amino acid residues and nucleobases involved in photoinduced cross-linking of DNA to histones and other proteins and in elucidating the chemical structures of the corresponding nucleobase–amino acid conjugates.

It is known that a number of proteins undergo cross-linking to DNA or oligodeoxyribonucleotides upon exposure to far-ultraviolet light; among these are the histones (Saito & Matsuura, 1985, and references cited therein; Kurochkina & Kolomiitseva, 1989, and references cited therein), *Escherichia coli* DNA polymerase I (Markovitz, 1972), RNA polymerase (Park et al., 1982), and DNA melting proteins, such as bovine pancreatic ribonuclease (Havron & Sperling, 1977), *E. coli* single-stranded DNA binding protein (Merrill et al., 1984), T4 phage gene 32 protein (Shamoo et al., 1988), and the A 1 heterogeneous nuclear ribonucleoprotein (Merrill et al., 1988). It has become clear that for significant cross-linking to occur, intimate interaction between the protein and nucleic acids is necessary; as a result, UV irradiation of such complexes can potentially provide a useful method for the study of the binding sites and sequence specificity of a variety of DNA-binding proteins. Studies by Shetlar et al. (1984a–c) have

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[1] Abbreviations: AtI, angiotensin I; CID, collision-induced dissociation; Dabsyl Cl, 4-(dimethylamino)azobenzene-4′-sulfonyl chloride; DCIMS, direct chemical ionization mass spectrometry; EIMS, electron ionization mass spectrometry; HPLC, high-performance liquid chromatography; LSIMS, liquid secondary ion mass spectrometry; NAT, *N*-acetyltyrosine; PABA, *p*-aminobenzoic acid; TSP, sodium (trimethylsilyl)propionate-2,2,3,3-$d_4$; TFA, trifluoroacetic acid; UV, ultraviolet.

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shown that a number of amino acids are capable of photobinding to DNA and homopolynucleotides of the naturally occurring nucleobases. It has often been assumed that the photoreactivity of the amino acids is dependent upon the nature of their side chains, and for this reason a number of studies have focused on using models representative of these side chains, such as aliphatic amines and alcohols [for reviews, see Shetlar (1980) and Saito and Sugiyama (1990)]. In the case of the nucleobases, the pyrimidines have attracted the greater attention, and a number of possible amino acid–pyrimidine base conjugates have been identified. These include thymine–cysteine conjugates (Varghese, 1973; Shetlar & Hom, 1987) and lysine conjugates with thymine, cytosine, and 5-methylcytosine (Saito et al., 1987, and references cited therein; Dorwin et al., 1988).

In the present paper, we focus on photoaddition reactions occurring in systems containing the nucleobase thymine and the tyrosyl moiety. Tyrosine (Tyr) is of considerable interest from the standpoint of understanding DNA–protein interactions. There exists a wealth of evidence demonstrating an important role for Tyr in the association of proteins and nucleic acids. Tyrosine and the other aromatic amino acids, tryptophan (Trp) and phenylalanine (Phe), are able to undergo stacking interactions with nucleic acid bases [see, for example, Anderson et al. (1975), Brun et al. (1975), and Mayer et al. (1979)]. This stacking interaction is strongly favored in interactions of proteins with single-stranded DNA. For example, Tyr is found in the DNA-binding regions of certain single-stranded nucleic acid binding proteins such as the gene 5 product of bacteriophage fd (O'Connor & Coleman, 1983) and gene 32 product of bacteriophage T4 (Prigodich et al., 1984, 1986). Photochemically, Tyr and related compounds are themselves quite reactive [for a review, see Creed (1984)]. When incorporated into proteins, however, the possibility of energy migration from Tyr to Trp must be considered, as such a process would tend to quench the reactivity of Tyr moieties. In proteins containing both Tyr and Trp, the fluorescent spectra obtained resemble that of Trp. This is explained by a 10-fold greater molar absorption coefficient for Trp than Tyr over the appropriate wavelength range and by efficient singlet and triplet energy transfer from Tyr to Trp, the limiting distance between chromophores being of the order of 6.3 nm (Grainger & Nishimura, 1977). (Both the lowest excited singlet and triplet energy levels are lower in energy in Trp than in Tyr.) Nevertheless, it has been shown that as much as 50% of the energy absorbed by Tyr can be retained, even when a Trp residue is close enough for energy transfer to occur (1.3 nm) (Menez et al., 1980). Furthermore, a number of biologically important DNA-binding proteins contain no Trp, in particular histones, and therefore quenching via energy transfer is greatly reduced.

Although Tyr is known to be able to photosensitize the formation of thymine cyclobutane dimers in DNA (Kaneko et al., 1979), relatively little is known about its photoreactive properties with any of the nucleobases of DNA. There is suggestive evidence in the literature, however, that such photoreactions could occur. An early survey study of the photoreactivity of thymine with the common amino acids indicated that Tyr is somewhat reactive toward photoconjugate formation with this nucleobase (Schott & Shetlar, 1974). A later survey of the photoreactivity of DNA and various homopolynucleotides of the nucleobases toward photoconjugate formation with the common amino acids, as well as the glycyl dipeptides of these amino acids, indicated that Tyr and glycyltyrosine form photoconjugates with these polymers with fairly high quantum efficiencies (Shetlar et al., 1984a–c). On the level of nucleic acid–protein complexes, there is not yet direct evidence implicating the participation of thymine–tyrosine conjugates in photo-cross-linking. However, it has been found that the uracil moiety, with a similar diketopyrimidine ring structure, is involved in the photo-cross-linking of 23*S* RNA to the L4 protein in the *E. coli* ribosome (Maly et al., 1980).

Irradiation of thymine or thymidine in solution with tyrosine (Tyr) and the closely related *N*-acetyltyrosine (NAT) leads to formation of conjugates between these compounds, along with the well-studied cyclobutane dimeric products of the nucleobase or nucleoside (Cadet & Vigny, 1990). In the present study, we have isolated and characterized these conjugates. We chose NAT for our initial work to eliminate possible photoreactions of the $\alpha$-amino group with thymine and thymidine and to facilitate chromatographic separations. We subsequently studied the photochemistry of Tyr in the presence of thymine and the photochemical reaction of thymidine with the Tyr-containing peptide angiotensin I. With the detailed information about the physical properties and chromatographic behavior of the conjugates obtained in these studies, it should be possible to test the hypothesis that similar adducts are involved in DNA–protein cross-linking in vitro and in vivo.

## MATERIALS AND METHODS

*General Aspects.* Far-ultraviolet irradiations were carried out in a cold room at 4 °C in a Vycor-shielded 650-mL quartz vessel placed in a Rayonet RPR-100 photoreactor equipped with Rayonet 2537 lamps emitting principally at 254 nm. All photolysis equipment was supplied by Southern New England Ultraviolet Co. All irradiations at $\lambda > 290$ nm were carried out using similar equipment; however, the vessel was Pyrex-shielded, and the photoreactor was equipped with Rayonet 3000 lamps. Oxygen was removed where necessary by bubbling 99.997% pure nitrogen for at least 30 min and then stoppering the vessel. UV absorption measurements were made using a Cary 118C spectrophotometer or a Hewlett-Packard HP8452A diode array spectrometer. High-resolution EIMS measurements were made using a Kratos MS9 mass spectrometer. Direct chemical ionization (DCI) molecular weight measurements were carried out on a Fisons/VG 70-250/VSE mass spectrometer using ammonia reagent gas. Peptide molecular weights were measured by LSIMS, using a Kratos MS-50S double-focusing mass spectrometer equipped with a high-field magnet. Tandem mass spectrometry experiments were performed on a Kratos Concept IIHH four-sector EBEB instrument (Walls et al., 1990) fitted with an electrooptical array detector, a cesium LSIMS source, and a cooled sample probe. Precursor ions were generated with an 18-keV $Cs^+$ primary ion beam. The collision energy for collision-induced dissociation (CID) was 4 keV. The collision gas (He) was used at a pressure sufficient to suppress the precursor ion beam to about 30% of its initial level. The instrument was controlled and data were acquired with a DS-90 data system. Data reduction and display were carried out on a Mach 3 data system. $^1H$ and $^{13}C$ NMR spectra were run on General Electric GE500 and QE300 spectrometers. NMR spectra were recorded in deuterated water, using TSP as internal reference. Proton chemical shifts were assigned by 2D COSY experiments, and the $^{13}C$ resonances were assigned by $^1H$–$^{13}C$ heteronuclear correlation spectroscopy.

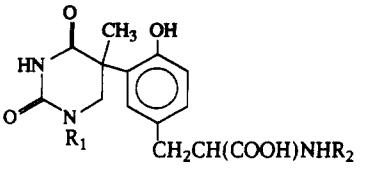
Photoproducts were purified by high-performance liquid chromatography (HPLC). The HPLC system consisted of

two Rainin Rabbit HP pumps, used in conjunction with a Dynamax data management package. Products were detected using a Kratos 783 variable-wavelength detector equipped with either a UV- or visible-range lamp, as required. Analytical work was effected using a Whatman Partisil 5 ODS-3 RaC II column, and preparative separations were carried out using a Whatman Partisil 5 ODS-3 RaC semi-preparative column of 10-cm length, packed with particles of 5-$\mu$m diameter. Desalting was achieved using a Hamilton RPR-1 column. Dabsyl–amino acid derivatives were separated using a 25-cm 5-$\mu$m Alltech Spherisorb reversed-phase column. All organic solvents were obtained from Fisher Scientific Co. Other chemicals were obtained from Sigma (thymine, thymidine, angiotensin I), Serva (NAT), and Fluka (2,4-dimethylphenol).

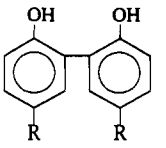
*Photochemistry in the Thymine–NAT System.* Generally, thymine/NAT solutions (500 mL, pH 8, 2 mM in each) were deoxygenated by nitrogen bubbling for 30 min. The samples were sealed and subsequently irradiated for 72 h. The irradiated solutions were then concentrated to 20 mL, acidified to pH 3 with 1 N HCl(aq), and made 20% in methanol. The thymine–NAT conjugate was isolated and purified by semi-preparative reversed-phase HPLC using 80/20 0.1% TFA/methanol as eluent. Using these conditions and the Whatman analytical ODS-3 column, the retention times were as follows: thymine, 3.1 min; adduct, 4.7 min; and NAT 5.4 min. The adduct (I) was characterized using a number of spectroscopic techniques, including one- and two-dimensional $^1$H and $^{13}$C NMR, LSIMS, and UV spectrophotometry. NMR data are presented in Tables I and II. The following additional spectroscopic data were obtained: UV ($H_2O$, pH 3.0) $\lambda_{max}$ 278 nm; LSIMS(+) $MH^+$ 350.

*Photochemistry in the Thymidine–NAT System.* The irradiation procedure was identical to that used in the thymine–NAT study described above. Owing to the instability of the osidic moiety of the nucleoside derivatives at low pH toward isomerization and anomerization, the HPLC conditions were modified. Instead of aqueous TFA/methanol eluents, a 2 mM phosphate buffer (pH 7.3) was used in conjunction with a Whatman C18 reversed-phase semipreparative column. At a flow rate of 4 mL/min, the adduct peak eluted after 14 min. The eluent was concentrated and the product was desalted on a Hamilton RP-1 column before final concentration and lyophilization. The single product peak was shown to consist of two diastereoisomers of product II. The isomers are distinguishable by high-field high-resolution $^1$H and $^{13}$C NMR; the NMR data are presented in Tables I and II. The following additional spectroscopic data were obtained: UV ($H_2O$, pH 3.0) $\lambda_{max}$ 278; LSIMS(+) $MH^+$ 466.

*Acid Hydrolysis of the Thymidine–NAT Adduct (II) To Form III.* The thymine–tyrosine analog of compounds I and II was also prepared via complete hydrolysis of II. Approximately 10 mg of II was dissolved in 3 mL of 1 N HCl. The solution was heated for 3 h at 100 °C in a heating block. The solution was then adjusted to pH 2.0 by addition of 1 N NaOH and made 10% in methanol. The thymine–tyrosine conjugate (III) was purified on the semipreparative Whatman Partisil 10 ODS-3 column using 0.1% TFA/methanol (90/10) at a flow rate of 4 mL/min; detection was by UV absorbance at 278 nm. Conversion was greater than 90% by HPLC peak intensities. The eluent was concentrated to 2 mL and lyophilized. The NMR data are presented in Tables I and II. The following spectroscopic data was also obtained: UV ($H_2O$, pH 7.2) $\lambda_{max}$ 278 nm; LSIMS(+) $MH^+$ 308, $[M + Na]^+$ 330.




I: $R_1$ = H, $R_2$ = $COCH_3$
II: $R_1$ = 2'-deoxyribosyl, $R_2$ = $COCH_3$
III: $R_1$ = H, $R_2$ = H




IV: R = $CH_2CH(COOH)(NHCOCH_3)$

*Photoreaction of Thymine with Tyrosine To Form III.* An aqueous solution of thymine/tyrosine (2 mM in each, 500 mL, pH 7.7) was deoxygenated by passage of nitrogen for 30 min, sealed, and irradiated at $\lambda$ = 254 nm for 100 h at 4 °C. The slightly brown solution was concentrated to 8 mL, filtered, acidified to pH 3.0 with 1 N HCl(aq), and chromatographed using the conditions described above. Using a Whatman ODS-3 RaC II column and 0.1% TFA/methanol (90/10) eluent at 1 mL/min, the retention times were as follows: thymine, 3.5 min; Tyr, 4.5 min; III, 5.4 min. The spectroscopic data for the sample of III via direct photolysis were identical to those obtained for the sample obtained by acid hydrolysis of II, as described in the previous section.

*Isolation of a Photodimer of NAT (IV).* A bityrosine derivative of NAT was found as a byproduct in all preparative irradiations of thymine–NAT and thymidine–NAT systems carried out under alkaline conditions. It was isolated chromatographically using isocratic 0.1% TFA/methanol (70/30). Using the analytical ODS3 RaC II column and a flow rate of 1 mL/min, the retention times in an irradiated thymidine/NAT system were as follows: thymidine, 2.4 min; NAT, 3.3 min; NAT dimer (IV), 6.7 min. The NMR data are presented in Tables I and II, and the following additional data were obtained: UV ($H_2O$, pH 4.1) $\lambda_{max}$ 284 nm; EIMS $m/z$ 444, $C_{22}H_{24}N_2O_8$; DCIMS $m/z$ 445 ($MH^+$).

*Photoreaction of Thymidine with Angiotensin I.* One milliliter of a solution, 2 mM in both thymidine and AtI (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) and 4 mM in sodium phosphate (pH 8.2), was placed in a cylindrical quartz vessel of 2-cm diameter. The neck of the vessel was sealed with a rubber sleeve stopper. Nitrogen gas, presaturated with water, was passed into the vessel through a needle, a second needle allowing escape of exhaust gas. The vessel was placed over a Vycor-shielded germicidal lamp. After passage of nitrogen over the solution for 1 h, the lamp was turned on and passage of nitrogen maintained throughout irradiation. At hourly intervals, 10-$\mu$L aliquots of the mixture were removed and analyzed by HPLC to determine the amount of remaining thymidine. After 3 h of irradiation, 60% of the thymidine had been lost and irradiation was discontinued. The photoreaction mixture was then run through a 0.22-$\mu$m MSI polyacetate filter to remove the small amount of precipitate present.

The irradiated mixture was separated by reversed-phase HPLC using an analytical Vydac C-18 column (0.46 × 25 cm). A linear gradient from 15 to 30% B over 40 min was used at a flow rate of 1.0 mL/min, where solvent A was 0.1%

TFA in water and solvent B was 0.08% TFA in acetonitrile. The absorbance of the HPLC eluate was monitored at 215 nm. Using these conditions, the adduct eluted after 26.7 min and parent AtI eluted at 28.4 min.

*Structural Analysis of the Thymidine–AtI Adduct.* Part of the eluent corresponding to the adduct, obtained as described above, was evaporated to dryness in a Pierce peptide hydrolysis tube. To the residue was added 400 μL of constant-boiling hydrochloric acid (Pierce). After the solution was cooled in dry ice, the tube was evacuated to 100 mTorr and sealed. The tube was placed in a heating block at 110 °C for 22 h, and the hydrolysis mixture was then blown to dryness with a stream of nitrogen. The residue was taken up in 200 μL of 0.1% TFA/methanol (90/10) and chromatographed under the conditions described above for the preparation of III. The peak that eluted with a retention time identical to that of III was collected and evaporated to dryness. The residue was analyzed, after dabsylation, via reversed-phase HPLC using a slight adaptation of the method of Vendrell and Avilés (1986). The residue was taken up in 50 μL of 0.1 M sodium bicarbonate (pH 8.35), and 50 μL of recrystallized Dabsyl Cl (27 mg/20 mL) in spectroscopic grade acetone was added. The solution was heated at 70 °C for 10 min with occasional shaking until the precipitate disappeared; 20 μL was then injected directly onto a Spherisorb column, using a 12 mM sodium phosphate (A)–acetonitrile (B) gradient for elution. The 14-min gradient used had the following profile: 18% B stepped to 20% B over 3 min, then stepped to 40% B over 6 min, then stepped to 70% B over 2 min, and held at 70% B for 3 min. An authentic sample of III was derivatized in the same way. Under the gradient conditions used, the diastereoisomers of the Dabsyl–III derivatives were separated. The Dabsyl derivatives of the sample of III obtained from hydrolysis of the thymidine–AtI adduct were shown to coelute with those derivatives prepared from authentic III.

The remainder of the product peak was analyzed by LSIMS, the spectrum of which showed an abundant ion at $m/z$ 1538.8, which corresponds to the expected (protonated) AtI–thymidine adduct. A second aliquot of this sample was analyzed by high-energy CID tandem mass spectrometry to verify the identity of the cross-linked amino acid.

*Estimation of the Quantum Yields for the Photoreactions of Thymine and Thymidine with NAT.* The general procedure for determination of quantum yields was similar to that described in detail in Shaw and Shetlar (1990). Stock thymine/NAT and thymidine/NAT solutions, 2 mM in each reactant, were prepared and buffered at pH 8 using 4 mM phosphate buffer. In separate experiments, a sample was placed in a 3-mL quartz cuvette with a magnetic bar stirrer and sealed with a septum. On each side of this cuvette, in equivalent positions next to a Vycor-shielded germicidal lamp, was placed a matched cuvette with bar stirrer containing the actinometer 1,3-dimethyluracil (3 mM), which undergoes a photohydration reaction with quantum yield of 0.0106. A needle was passed through the septum of the NAT–thymine (or thymidine) sample, which was connected to a nitrogen tank. A second short needle passed through the septum to allow escape of nitrogen. The sample was bubbled with nitrogen with constant stirring for 45 min, after which time the three samples were irradiated next to Vycor-shielded germicidal lamp for 1 h. (Irradiation for this length of time results in loss of only a small percentage of the parent compound.) Nitrogen bubbling and stirring were continued throughout the irradiation. The quantum yields were determined chromatographically, using HPLC conditions described above for analysis of the thymine–NAT (or thymidine–NAT) conjugates and the conditions described in Shaw and Shetlar (1990) for analysis of the actinometric product. For each individual run, the concentrations of adduct and of the photohydrate of 1,3-dimethyluracil were determined from standard curves of chromatographic peak area vs concentration. Since the molar absorption coefficients of the adducts could not be determined accurately, the value was approximated using a commercially obtained sample of 2,4-dimethylphenol. The $\epsilon$ obtained for this sample in the HPLC eluent used (0.1% TFA/methanol, 80/20, pH 2.0) was found to be about 2140 $M^{-1}$ $cm^{-1}$ at $\lambda_{max}$ = 278 nm.

The variability between repeat determinations of the quantum yield for adduct formation was always found to be significant in both thymine–NAT and thymidine–NAT systems. For example, in a pair of runs on the T–NAT system made at pH 7.8, values of $4.1 \times 10^{-4}$ and $5.5 \times 10^{-4}$ were obtained for the quantum yield for formation of I, while in the dT–NAT system, a pair of runs at pH 8.1 gave corresponding values of $1.5 \times 10^{-3}$ and $2.3 \times 10^{-3}$. The likely cause of this variability is sensitivity of the photoreaction rate to the presence of small amounts of oxygen (see below). If this is indeed the case, then the values of $5.5 \times 10^{-4}$ and $2.3 \times 10^{-3}$ for compounds I and II, respectively, can be considered lower limits to the quantum yields at pH values near 8.

*Effects of Oxygen on Photoadduct Formation.* Two samples of thymidine–NAT were prepared as described above for the quantum yield study. One sample was deoxygenated by passage of nitrogen for 1 h before and throughout irradiation, while the second sample was left saturated with air. The yield of the thymidine–NAT adducts was determined chromatographically in the usual way. Under these conditions, the formation of II was completely quenched by the presence of the oxygen in the air.

*Photoreaction in the Thymidine–NAT System at $\lambda > 290$ nm.* Samples of thymidine–NAT, 2 mM in each and in phosphate buffer (4 mM, pH 8.2), were irradiated in deoxygenated solution as above, using a Pyrex filter that cut out wavelengths less than 290 nm. The same adducts were produced as when irradiations were done with the Vycor-shielded lamp; however, because of the smaller amount of light absorbed by the system under these conditions, the rate of adduct formation was slower than was the case when the Vycor filter was used.

*pH Dependence of the Quantum Efficiency for the Photoreactions of Thymine and Thymidine with NAT.* The quantum yield for formation of photoadducts was found to be strongly pH dependent. The quantum yields for each system over the pH range 4.0–9.5 were determined as follows. Solutions were prepared as above at various pH values differing by half-pH units, and 3-mL samples corresponding to each pH were placed in 15-mL quartz tubes. These solutions were degassed by passage of nitrogen for 45 min and the tubes then stoppered and sealed. The tubes were placed in a carousel in the Rayonet photoreactor and irradiated at 254 nm for 1 h at 4 °C. The samples were acidified to pH 2 by addition of 5 μL of concentrated HCl and analyzed by HPLC using a Whatman Partisil ODS-3 RaC II column and 0.1% TFA/methanol eluent; detection was at 278 nm. The quantum yield vs pH curve was obtained by relating the detector response for the pH 8.0 sample to the known quantum yield determined above and a concentration/detector response calibration curve obtained for the adduct. Figure 1 shows the curves obtained for the thymine–NAT and thymidine–NAT adduct formation. As stated above, the results given should be taken as

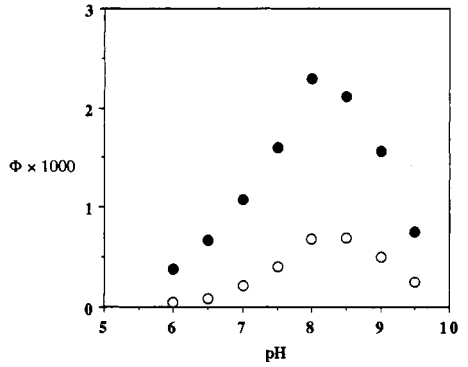



FIGURE 1: pH profiles for Φ, the quantum yields for the formation of *N*-acetyltyrosine adducts with thymine and thymidine. Samples were 2 mM in each reactant, and solutions were degassed prior to irradiation. The open circles correspond to the thymine–NAT system, while the solid circles describe the thymidine–NAT system.

approximate in view of the high oxygen sensitivity of the quantum yield and the approximated molar extinction coefficient for the adducts.

*Acetone Photosensitization Studies.* Three samples of thymidine–NAT, approximately 1.6 mM in each component and buffered with phosphate at pH 8.0, were prepared. One sample was acetone-free; the remaining samples contained acetone at concentrations of 10 and 20% (v/v). The solutions, contained in 3-mL quartz cuvettes, were degassed by passage of nitrogen for 45 min, as before, and irradiated using a lamp emitting primarily at $\lambda > 290$ nm, which was surrounded by a Pyrex filter with an effective cutoff at 290 nm. Above this wavelength, it was determined that at 10% concentration the acetone was absorbing 20 times as much light as the thymidine–NAT. The samples were analyzed by HPLC as described above.

## RESULTS AND DISCUSSION

*Elucidation of the Photoadduct Structures.* The isolated thymine–NAT and thymidine–NAT adducts were extensively characterized by a number of spectroscopic techniques. In each case, there were two isomers present in the isolated peak, corresponding to the 5*R* and 5*S* configurations. For the thymine–NAT adduct, the two isomers coelute exactly on all HPLC systems tried; the thymidine–NAT isomers could only be distinguished using the analytical ODS-3 column, in which case there was significant overlap between the corresponding peaks. Under preparative-scale conditions, the peaks corresponding to the isomers were indistinguishable and the products were analyzed spectroscopically as mixtures. The mass spectra of the mixtures gave the expected molecular weights. The UV absorption spectra were consistent with those expected for substituted phenols attached to a dihydrothymine residue. The wavelength maximum in the UV profile for the adducts at pH 2 was identical to that obtained for 2,4-dimethylphenol, which has the same substitution pattern as the adducts. The most detailed structural information was obtained from high-field $^1$H and $^{13}$C NMR spectra. The data are presented in Tables I and II. The 500-MHz $^1$H NMR spectrum of the nucleoside products provided sufficient resolution for the analysis of the spectra of the diastereoisomers individually. The proton connectivities were established by a 2D COSY experiment. The thymine base and amino acid moieties for both the thymine and thymidine adducts were similar in each case. The coupling patterns for the aromatic proton signals indicated a trisubstituted phenyl compound. Comparison of the chemical shift and coupling patterns recorded for these compounds with those recorded for 2,4-dimethylphenol and those reported for the uracil adduct to Tyr (obtained via UV photolysis of 5-bromouracil with tyrosine) by Dietz and Koch (1987) indicated that the thymine residue was attached ortho to the hydroxyl group. The photoreactions of thymine and thymidine with *p*-aminobenzoic acid (PABA) to form analogous products were reported by us recently (Shaw et al., 1992), and the comparison of the spectroscopic data of these products with those studied in the present paper lend further support to their structural assignment. The 5,6-saturation of the thymine moiety is evidenced by the C6 AB quartet. The geminal protons are magnetically nonequivalent, being separated by 1.75 ppm. This has been reported for other 5,6-dihydrothymine compounds substituted at C5 by groups where aromatic ring currents or electron-withdrawing groups affect one geminal proton more than the other (Berger et al., 1986; Shaw et al., 1992). For the nucleoside, the coupling patterns and chemical shifts of the osidic proton resonances are consistent with other 2′-deoxy-β-D-*erythro*-pentafuranosylthymine derivatives. The $^{13}$C NMR data again showed the presence of two isomers. The chemical shifts for the two isomers are indistinguishable for a number of signals and at most only differ by 0.3 ppm. The hydrogen-bearing carbon signals were assigned by a 2D heteronuclear correlation experiment, and the quaternaries were assigned by comparison with those of NAT and 2,4-dimethylphenol.

The AtI–thymidine adduct was characterized by mass spectrometry as well as by the acid hydrolysis described above. The tandem CID spectrum of the modified peptide is shown in Figure 2. This spectrum shows that the sequence of the adduct is identical to that of AtI except that the mass of the former Tyr residue has been increased by 242 Da, which is equal to the mass of deoxythymidine. Other peaks in the spectrum (marked $MH^+$-dThd, -R′X, $a_5$-dThd, and $a_6$-dThd) correspond to side-chain losses from the modified Tyr structure and to the expected immonium ion (marked X). The tandem mass spectrometric results show unequivocally that, under the conditions used, the cross-link is formed exclusively to Tyr and not to any of the other amino acid residues present in AtI.

Minor HPLC peaks were also observed. One corresponded to a species that had a monoisotopic mass (2590.2) equaling twice that of AtI less two hydrogens, while the second, with a monoisotopic mass of 2832.4, corresponds to the same type of dimeric species but incorporating a thymidine residue. The first minor product is likely a dimer of AtI produced by formation of an analog of bityrosine; it can be speculated that the second incorporates a bitryosine moiety in which one of the tyrosyl residues has formed a photoadduct with thymidine. However, detailed structural characterization of these species has not been undertaken.

*Mechanistic Considerations.* The formation of the thymine–NAT and thymidine–NAT adducts occurs when mixtures are irradiated both at 254 nm and wavelengths >290 nm. Both the amino acid and the thymine/thymidine absorb significantly in each case. However, the mechanism is probably similar to that reported for formation of the analogous thymine– and thymidine–PABA adducts (Shaw et al., 1992). This involves photoionization of NAT to yield the radical cation, which should deprotonate rapidly ($pK_a$ about 2.0; Dixon & Murphy, 1976) to form the corresponding oxygen-centered radical. The ejected electron is likely to be captured by thymine or thymidine to yield a radical anion, which then protonates at C6 to produce the C5-yl radical. Combination of these two radicals would then yield the observed adducts. Addition of

Table I: $^1$H NMR Data for Compounds I–IV[a]

| | Ha | Hb | Hc | Hα | H6(a) | H6(b) | Hβ(1) | Hβ(2) | $CH_3CO$ | $CH_3$ | H1′ | H2′ | H2″ | H3′ | H4′ | H5′ | H5″ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 6.86 | 7.13 | 7.24 | 4.63 | 4.92 | 3.16 | 3.16 | 2.96 | 1.95 | 1.67 | | | | | | | |
| | 6.86 | 7.12 | 7.23 | 4.61 | 4.91 | 3.16 | 3.14 | 2.96 | 1.96 | 1.67 | | | | | | | |
| II | 6.86 | 7.13 | 7.25 | 4.42 | 3.99 | 3.22 | 3.13 | 2.88 | 1.94 | 1.68 | 6.30 | 2.30 | 2.16 | 4.29 | 3.84 | 3.72 | 3.64 |
| | 6.87 | 7.14 | 7.19 | 4.41 | 4.01 | 3.14 | 3.14 | 2.89 | 1.95 | 1.66 | 6.26 | 2.07 | 1.99 | 4.27 | 3.90 | 3.63 | 3.53 |
| III | 6.93 | 7.21 | 7.30 | 4.24 | 3.96 | 3.06 | 3.30 | 3.18 | | 1.71 | | | | | | | |
| | 6.91 | 7.17 | 7.33 | 4.19 | 3.96 | 3.06 | 3.28 | 3.15 | | 1.70 | | | | | | | |
| IV | 6.94 | 7.17 | 7.02 | 4.61 | 3.15 | 2.96 | | | 1.95 | | | | | | | | |

| | $J_{ab}$ | $J_{bc}$ | $J_{\alpha\beta(1)}$ | $J_{\alpha\beta(2)}$ | $J_{\beta gem}$ | $J_{6gem}$ | $J_{1'2'}$ | $J_{1'2''}$ | $J_{2'2''}$ | $J_{2'3'}$ | $J_{2''3'}$ | $J_{3'4'}$ | $J_{4'5'}$ | $J_{4'5''}$ | $J_{5'5''}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 8.2 | 2.2 | 5.7 | 8.6 | −14.1 | −12.7 | | | | | | | | | |
| | 8.2 | 2.2 | 5.6 | 8.5 | −14.1 | −12.7 | | | | | | | | | |
| II | 8.1 | 2.1 | 4.6 | 8.7 | −14.0 | −12.6 | 7.6 | 6.7 | −14.1 | 6.8 | 3.9 | 4.2 | 4.0 | 5.5 | −12.3 |
| | 8.1 | 2.1 | 4.7 | 8.5 | −14.1 | −12.5 | 8.9 | 6.5 | −14.2 | 6.6 | 3.6 | 3.3 | 4.2 | 5.7 | −12.1 |
| III | 8.2 | 2.2 | 5.5 | 7.2 | −14.7 | −12.7 | | | | | | | | | |
| | 8.2 | 2.2 | 5.5 | 7.8 | −14.6 | −12.7 | | | | | | | | | |
| IV | 8.3 | 2.2 | 5.6 | 8.5 | −14.0 | | | | | | | | | | |

[a] Chemical shift data (δ) and coupling constants (Hz) were determined in $D_2O$ against TSP internal standard. For compounds I–III, two sets of data are presented corresponding to the 5*R** and 5*S** diastereoisomers

Table II: $^{13}$C NMR Chemical Shift Data for Compounds I–IV Recorded in $D_2O$[a]

| | $COCH_3$ | Cβ | Cα | C1 | C2 | C3 | C4 | C5 | C6 | $COCH_3$ | $CO_2H$ | $CH_3$ | C2 | C4 | C5 | C6 | C1′ | C2′ | C3′ | C4′ | C5′ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 22.4 | 36.8 | 55.0 | 156.3 | 127.2 | 129.5 | 129.1 | 131.0 | 117.2 | 174.7 | 175.7 | 20.7 | 153.3 | 179.8 | 44.5 | 47.4 | | | | | |
| | 22.4 | 36.8 | 55.1 | 156.3 | 127.3 | 129.6 | 129.1 | 131.1 | 117.3 | 174.7 | 175.8 | 20.7 | 153.3 | 179.8 | 44.5 | 47.3 | | | | | |
| II | 22.7 | 35.9 | 57.3 | 154.4 | 126.2 | 130.4 | 129.4 | 131.1 | 117.2 | 173.9 | 178.9 | 20.1 | 153.1 | 179.1 | 45.1 | 47.0 | 84.6 | 37.8 | 71.6 | 85.9 | 62.4 |
| | 22.8 | 36.2 | 57.4 | 155.0 | 126.5 | 130.5 | 129.5 | 131.1 | 117.2 | 174.0 | 179.0 | 20.5 | 153.2 | 179.1 | 45.1 | 47.0 | 84.7 | 37.9 | 71.7 | 86.0 | 62.4 |
| III | | 35.9 | 55.8 | 156.2 | 126.9 | 130.0 | 127.8 | 131.5 | 117.8 | | 173.1 | 20.7 | 154.1 | 180.1 | 44.4 | 47.2 | | | | | |
| | | 36.0 | 55.6 | 156.2 | 126.7 | 129.9 | 127.7 | 131.4 | 117.7 | | 173.1 | 20.7 | 154.1 | 180.1 | 44.4 | 47.2 | | | | | |
| IV | 22.4 | 37.6 | 55.3 | 156.2 | 127.2 | 130.5 | 129.9 | 133.5 | 117.4 | 174.8 | 173.3 | | | | | | | | | | |

[a] *p*-Dioxane was used as internal standard (*p*-dioxane = TMS + 67.4 ppm). Two entries for certain signals indicate where the corresponding resonances for the individual isomers are resolved. No assignment of particular signals to given isomers could be made because of the very small differences in chemical shift.

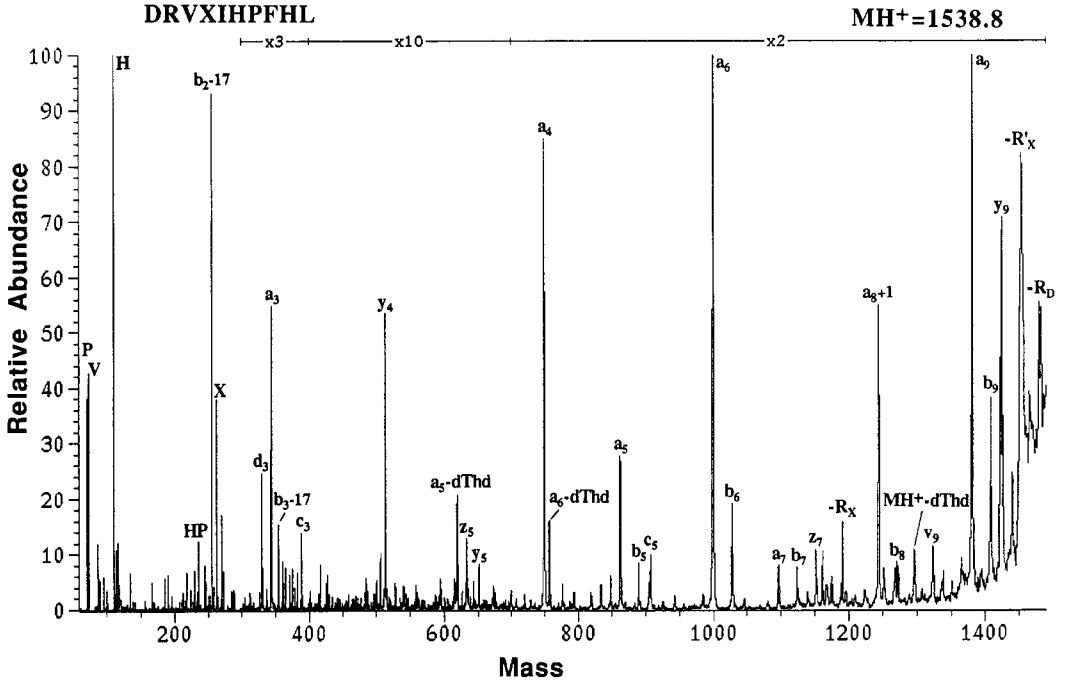



FIGURE 2: High-energy CID spectrum of modified AtI. Peaks labeled with lower case letters correspond to sequence ions, upper case letters denote immonium ions, and –R indicates a side-chain loss from the protonated molecule. Peak designations conform to the conventions described in Biemann (1990). Other peak labels are described in the text.

the thyminyl radical to the position on NAT ortho to the hydroxyl group is reasonable; the resonance form with reduced spin-paired density at this position appears to be the most likely to undergo combination, again by analogy with the results of the PABA investigation of Shaw et al. (1992). The fact that both the 5*S* and 5*R* diastereomeric forms of the nucleobase–amino acid photoadducts were produced in these reactions (see above) is consistent with the above mechanistic pathway.

Bityrosine, which is analogous to IV, is a well-documented Tyr photolysis product (Malencik et al., 1990, and references cited therein). These compounds are undoubtedly the result of combination of two phenoxyl radicals in the ortho resonance form. Dietz and Koch (1987) reported the formation of

bityrosine during UV irradiation of mixtures of 5-bromouracil with Tyr.

The complete quenching of nucleobase–amino acid adduct formation in the presence of oxygen again parallels observations made in studies of the photoreactions of thymine and thymidine with PABA (Shaw et al., 1992). Oxygen is able to quench triplet states, as well as react with the thymine radical anion through electron transfer or addition at radical sites. In particular, oxygen has been shown to efficiently quench phenolic triplet states, including that of Tyr, by an electron-transfer mechanism with the formation of phenoxyl radicals and superoxide radicals (Bent & Hayon, 1975). Although the phenoxyl radical is an intermediate in the proposed mechanism, electron transfer to oxygen would preempt formation of the other proposed intermediate, the thymine radical anion. In addition, oxygen is also able to efficiently quench the thymine radical anion (Theard et al., 1971).

Acetone is widely used to test the possible involvement of triplet states in photochemical reactions, since the quantum efficiency for intersystem crossing to the acetone triplet state is close to 1.0 (Murov, 1973). Since, under the experimental conditions used in our study, acetone absorbs the major proportion of light at wavelengths above 290 nm, a reduction in the yield of adduct would be observed if a triplet state is not involved. We determined that in a 10% solution of acetone, 2 mM each in thymidine and NAT, acetone absorbs 95% of the light above 290 nm. Thus, the absence of triplet-state sensitization would be expected to produce a yield of adduct that was 5% of that observed in the absence of acetone. In fact, we detected a 15% yield. However, it was clear that a significant amount of acetone had been lost during the photoreaction due to the degassing procedure, and this could possibly account for the higher than expected yield. To confirm this, we repeated the experiment using 20% acetone and observed a reduction in yield to 7.5% of that found in the absence of acetone. This strongly suggests that the reaction is not photosensitized by the presence of acetone. Ordinarily, this would be strong evidence that the photoreaction does not proceed via a triplet-state intermediate. However, a study conducted by Catalani and Bechara (1984) showed that Tyr does not interact with acetone triplet states at a diffusion controlled rate; indeed, the observed rate of quenching of acetone triplets is 1–2 orders of magnitude slower than the expected collisional rate. Since thymine and thymidine are known to quench the acetone triplet state (Cadet & Vigny, 1990), it is likely that a significant amount of the triplet-state energy transfer occurs to thymine and thymidine. The results thus strongly suggest that thymine and thymidine triplet states are not involved in photoadduct formation, in accord with our proposed mechanism; however, based on our present results, we are unable to rule out the involvement of a NAT triplet state in this reaction.

The mechanism of the photoionization of phenol and its derivatives, and in particular tyrosine, has been studied extensively [for a review, see Creed (1984)]. (Such photoionization corresponds to the first step in our proposed mechanism for photoadduct formation.) Flash photolysis studies have yielded strong evidence that, at neutral pH, photoionization is the result of biphotonic processes with absorption of a second photon by the Tyr triplet. At pH 12, where the Tyr is in the phenolate form, photoionization in monophotonic. In the case of steady-state irradiation, the evidence to date suggests that the photoionization process is quite different from that suggested by flash photolysis studies.

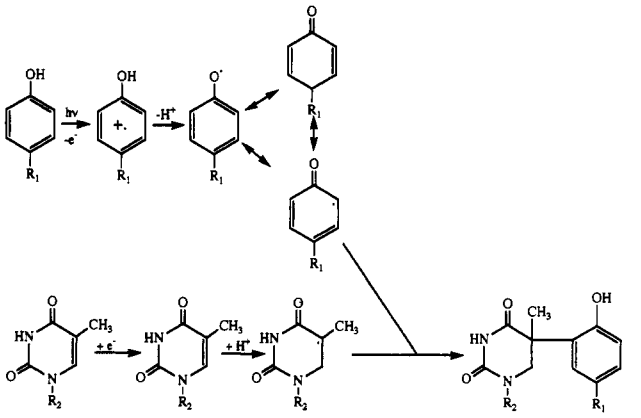


FIGURE 3: Proposed mechanism for the UV-induced formation of the thymine–tyrosine adduct.

At neutral pH, the intermediate appears to be the Tyr singlet state and the process is monophotonic. There is evidence that excited Tyr and phenol form exciplexes with water, and these may be precursors to electron loss. In the case of the photoreaction under study here, the question of the nature of the photoionization step must be regarded as open.

The mechanism outlined in Figure 3 describes stepwise formation of the thymine and NAT radical intermediates which then combine to yield products. However, it is also possible that electron and proton transfer to thymine occurs directly from excited NAT in a solvent cage. The excited-state NAT may be quenched by an associated thymine or thymidine in a manner similar to that of oxygen (Bent & Hayon, 1975), accompanied by rapid radical combination.

The pH dependence of the photoadduct formation requires comment. There are a number of possible explanations for the downturn in the magnitude of the quantum yield for adduct formation at pH values above 8.5. The amount of the bityrosine product of NAT was determined during our study of the pH dependence of the quantum yield for formation of I and II and was found to increase dramatically above pH 7–7.5; this suggests that formation of this compound may constitute a competing reaction pathway for the NAT phenoxyl radical at high pH. Flash photolysis studies (Bent & Hayon, 1975) have reported on the effects of pH on the yields for various excited-state reactions. According to these studies, the yield of $e^-$(aq) remained constant over the range of pH values used in our studies. However, the yield of triplet states decreased steadily over the range of pH 8–12. These observations, taken together, could be suggestive of the involvement of both singlet and triplet states in the photoionization process. However, in view of the inadequacy of our present knowledge of the photochemistry of tyrosine, it is difficult to say more about the reasons behind the observed pH effects. From the biological perspective, perhaps the most important features of the pH profiles for adduct formation are the significant yields in the range of physiological pH values.

*Conclusions.* We have isolated and characterized photoconjugates of thymine and thymidine with *N*-acetyltyrosine and of thymine with Tyr. These conjugates each possess a bond from the aromatic ring of the amino acid to C5 of the 5,6-saturated nucleobase or nucleoside. The mechanism of formation is proposed to involve production of a radical cation on the tyrosyl residue and a radical anion on the thymine moiety, followed by proton transfer and radical combination reactions. We have also demonstrated that the same type of reaction occurs when a decapeptide (angiotensin I) containing a single tyrosine is irradiated in the presence of thymidine.

The resulting adduct was isolated chromatographically, and the position of thymidine attachment was determined to be on the Tyr by tandem mass spectrometry; the identity of the thymine–tyrosine photoadduct formed after acid digestion with that formed in the analogous reactions of thymine and Tyr was verified by derivatization and cochromatography experiments. The application of mass spectral sequencing of peptides modified by conjugation with nucleobase moieties, such as accomplished in this work, promises to be a valuable technique for future photochemical studies of DNA–protein interactions.

Since the photoadditions between the thymine and Tyr moieties, described above, occur both with 254-nm light and with light of $\lambda > 290$ nm, it seems possible that this type of photoconjugate participates in the cross-linking of proteins to DNA that is observed when DNA–protein complexes are irradiated with either UVC or UVB light.

## REFERENCES


Anderson, R. A., & Coleman, J. E. (1975) *Biochemistry 14*, 5485–5491.

Bent, D. V., & Hayon, E. (1975) *J. Am. Chem. Soc. 97*, 2599–2606.

Biemann, K. (1990) *Methods Enzymol. 193*, 886–887.

Brun, F., Toulmé, J.-J., & Hélène, C. (1975) *Biochemistry 14*, 558–563.

Budowsky, E. I., & Abdurashidova, G. G. (1989) *Prog. Nucleic Acid Res. Mol. Biol. 37*, 1–65.

Cadet, J., & Vigny, P. (1990) in *Bioorganic Photochemistry* (Morrison, H., Ed.) Vol. I, pp 1–272, Wiley, New York.

Catalani, L. H., & Bechara, E. J. H. (1984) *Photochem. Photobiol. 39*, 823–830.

Creed, D. (1984) *Photochem. Photobiol. 39*, 563–575.

Dietz, T. M., & Koch, T. H. (1987) *Photochem. Photobiol. 46*, 971–978.

Dixon, W. T., & Murphy, D. (1976) *J. Chem. Soc., Faraday Trans. 2*, 1221–1230.

Dorwin, E. L., Shaw, A. A., Hom, K., Bethel, P., & Shetlar, M. D. (1988) *J. Photochem. Photobiol. B 2*, 265–278.

Dreyfuss, G., Choi, Y. D., & Adam, S. A. (1984) *Mol. Cell. Biol. 4*, 1104–1114.

Friedberg, E. C. (1985) *DNA Repair*, Freeman, New York.

Grainger, D. A., & Nishimura, A. M. (1977) *Biophys. J. 20*, 383–386.

Havron, A., & Sperling, J. (1977) *Biochemistry 16*, 5631–5635.

Hruska, F. E., Berger, M., & Cadet, J. (1985) *Can. J. Chem. 63*, 15–23.

Kaneko, M., Matsuyama, A., & Nagata, C. (1979) *Nucleic Acids Res. 6*, 1177–1186.

Kurochkina, L. P., & Kolomiitseva, G. Ya. (1989) *Anal. Biochem. 178*, 86–92.

Malencik, D. A., Zhao, Z., & Anderson, S. R. (1990) *Anal. Biochem. 184*, 353–359.

Maly, P., Rincke, J., Ulmer, E., Zwieb, C., & Brimacombe, R. (1980) *Biochemistry 19*, 4179–4188.

Markovitz, A. (1972) *Biochim. Biophys. Acta 281*, 522–534.

Mayer, R., Toulmé, F., Montenay-Garestier, T., & Hélène, C. (1979) *J. Biol. Chem. 254*, 75–82.

Menez, A., Montenay-Garestier, T., Fromageot, P., & Hélène, C. (1980) *Biochemistry 19*, 5202–5208.

Merrill, B. M., Williams, K. R., Chase, J. W. & Konigsberg, W. H. (1984) *J. Biol. Chem. 259*, 10850–10856.

Merrill, B. M., Stone, K. L., Cobianchi, F., Wilson, S. H., & Williams, K. R. (1988) *J. Biol. Chem. 263*, 3307–3313.

Murov, S. L. (1973) in *Handbook of Photochemistry*, p 3, Dekker, New York.

Myles, G. M., & Sancar, A. (1989) *Chem. Res. Toxicol. 2*, 197–226.

O'Connor, T. P., & Coleman, J. E. (1983) *Biochemistry 22*, 3375–3381.

Park, C. Z., Hillel, Z., & Wu, C.-W. (1982) *J. Biol. Chem. 257*, 6944–6949.

Peak, M. J., & Peak, J. G. (1989) *Photodermatology 6*, 1–15.

Prigodich, R. V., Casas-Finet, J., Williams, K. R., Konigsberg, W. H., & Coleman, J. E. (1984) *Biochemistry 23*, 522–529.

Prigodich, R. V., Shamoo, Y., Williams, K. R. Chase, J. W., Konigsberg, W. H., & Coleman, J. E. (1986) *Biochemistry 25*, 3666–3672.

Saito, I., & Matsuura, T. (1985) *Acc. Chem. Res. 18*, 134–140.

Saito, I., & Sugiyama, H. (1990) in *Bioorganic Photochemistry* (Morrison, H., Ed.) Vol. I, pp 317–340, Wiley, New York.

Saito, I., Sugiyama, H., Ito, S., Furukawa, N., & Matsuura, T. (1981) *J. Am. Chem. Soc. 103*, 1598–1600.

Schott, H. N., & Shetlar, M. D. (1974) *Biochem. Biophys. Res. Commun. 59*, 1112–1116.

Shamoo, Y., Williams, K. R., & Konigsberg, W. H. (1988) *Proteins 4*, 1–6.

Shaw, A. A., & Shetlar, M. D. (1990) *J. Am. Chem. Soc. 112*, 7736–7742.

Shaw, A. A., Wainschel, L. A., & Shetlar, M. D. (1992) *Photochem. Photobiol. 55*, 657–663.

Shetlar, M. D. (1980) *Photochem. Photobiol. Rev. 5*, 105–197.

Shetlar, M. D., & Hom, K. (1987) *Photochem. Photobiol. 45*, 703–712.

Shetlar, M. D., Christensen, J., & Hom, K. (1984a) *Photochem. Photobiol. 39*, 125–134.

Shetlar, M. D., Hom, K., Carbone, J., Moy, D., Steady, E., & Watanabe, M. (1984b) *Photochem. Photobiol. 39*, 135–140.

Shetlar, M. D. Carbone, J., Steady, E., & Hom, K. (1984c) *Photochem. Photobiol. 39*, 141–144.

Theard, L. M., Peterson, F. C., & Myers, L. S. (1971) *J. Phys. Chem. 75*, 3815–3821.

Varghese, A. J. (1973) *Biochemistry 12*, 2725–2730.

Walls, F. C., Baldwin, M. A., Falick, A. M., Gibson, B. W., Gillece-Castro, B. L., Kaur, S., Maltby, D. A., Medzihradszky, K. F., Evans, S., & Burlingame, A. L. (1990) in *Biological Mass Spectrometry* (Burlingame, A. L., & McCloskey, J. A., Eds.) pp 197–214, Elsevier, Amsterdam.

Wang, S. Y., Ed. (1976) *Photochemistry and Photobiology of Nucleic Acids*, Vol. 1 and 2, Academic Press, New York.