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Ultraviolet-induced 8,8-adenine dehydrodimers in oligo- and polynucleotides

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ABSTRACT

Characteristic fluorescence excitation and emission is induced by either acetone-sensitized 313 nm irradiation of mixtures of 8-bromoadenosine and adenosine or 254 nm irradiation of oligo- and polynucleotides containing adenine neighbors. The acetone-sensitized reaction involves cleavage of bromine from 8-bromoadenosine with activation of C-8, leading to formation of an 8,8-adenosine dehydrodimer. Comparable fluorescence properties arise in the unsensitized photoreaction of dApdA, pdApdA, ApA, poly(dA), poly(A), poly(dA·dT), and poly(dA·U). The previously unidentified adenine ultraviolet photoproduct described by Porschke has been isolated as several variants from solutions of pdApdA and poly(dA) irradiated at 254 nm. Based upon fluorescence spectra and mass spectra, these variants are shown to contain the 8,8-adenine dehydrodimer moiety.

INTRODUCTION

An unidentified ultraviolet-absorbing photoproduct formed upon irradiation at 254 nm of pdApdA or poly(dA) was first described by Porschke (1). Later, 8,8-purine ribonucleoside dehydrodimers were shown to be formed upon irradiation at 313 nm of mixtures of 8-Br-purine nucleosides and purine nucleosides in the presence of acetone as a photosensitizer (2,3). In the present study, based principally upon fluorescence excitation and emission spectra, it is shown that photoproducts with the same fluorescence characteristics as the 8,8-Ado dehydrodimer form in solutions of dApdA, pdApdA, ApA, poly(dA), poly(A), poly(dA·dT) and poly(dA·U) irradiated at 254 nm. This identity, in conjunction with ultraviolet absorbance and mass spectral data for some of the isolated photoproducts which correspond to that described by Porschke, lead to the conclusion that the Porschke photoproduct contains the same dehydrodimer moiety formed from adjacent adenine residues. The kinetics of photoproduct formation are shown to depend upon the composition and conformation of the strand in which these residues are located. Differences in the fluorescence properties of the dehydrodimer formed

after different extents of irradiation or present in different isolated fractions are accounted for in terms of variations in the microenvironment of the dimerized base moieties that arise when glycosyl or phosphodiester bonds strained by formation of the photoproduct are broken, making possible altered stacking of the aromatic rings. A preliminary report of these findings has been given (4).

MATERIALS AND METHODS

Chemicals. pdApdA, dApdA, dGpdA, dGpdG, 8-BrAdo, Ado, 8-BrAMP, AMP, ApA, poly(dAdT) and poly(dT) (Sigma) were used without further purification. Poly(dA) was a gift from Professor F. J. Bollum. Poly(U) and poly(A) were synthesized with polynucleotide phosphorylase (5).

Solutions. Poly(dA·dT) and poly(dA·U) were prepared for irradiation by mixing equimolar residue amounts of each strand in 0.1 M NaCl, 0.01 M Na_2HPO_4, 0.2 mM EDTA, pH 7.2. Poly(dAdT) was annealed in the same buffer prior to irradiation to assure complete double helical structure free of hairpins. Poly(dA) and poly(A) were irradiated in the same buffer. All monomers and oligomers were irradiated in d.H_2O with or without 30 % acetone.

Enzymatic hydrolysis. An irradiated solution of poly(dA) (150 μ g) was lyophilized, the residue was resuspended in 0.3 ml 15 mM sodium acetate, pH 7, and 20 units of bovine spleen phosphodiesterase I (Sigma) were added. After 24 h at 37° C, 20 more units were added and incubation was continued for another 24 h. The pH was then adjusted to 8, 5 units of bovine intestine alkaline phosphatase (Sigma) were added, and the mixture was incubated for 24 h. at 37° C.

Chromatography. The Porschke photoproduct was directly isolated (without enzymatic digestion and removal of phosphates) from irradiated solutions of pdApdA using an ion exchange HPLC column (SAX, Analytichem, 250 x 4.6 mm) (see Figure 6 for details).

The photoproducts from irradiated poly(dA) were isolated by applying the enzymatic digest to an octyldecylsilane (ODS) reversed phase HPLC column (Ultrasphere, Altex, 250 x 4.6 mm) (see Figure 8 for details).

Fluorescence emission was used to directly locate and characterize the photoproducts eluted from these columns. After lyophilizing, the desired poly(dA)-digest fractions were redissolved in H_2O and desalted by passage through a Biogel P-2 column in preparation for mass spectral analysis.

Spectroscopy. Photoproduct formation was monitored using ultraviolet and fluorescence spectroscopy. Ultraviolet spectra were recorded on a Cary 14

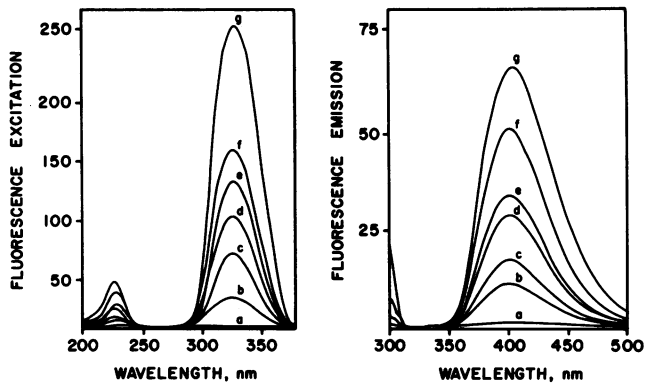


Figure 1. Excitation (left) and emission (right) spectra developed upon acetone-sensitized irradiation at 313 nm of an equimolar mixture of 8-BrAdo and Ado (each 0.30 mM) in 30 % acetone/H_2O. Minutes of irradiation: a) 0, b) 100, c) 200, d) 300, e) 542 and f) 1348.

spectrophotometer with the cell compartment maintained at 25° C.

Routine fluorescence excitation and emission spectra were recorded on a Perkin-Elmer MPF-4 spectrofluorimeter. Samples were maintained at 25° C. Individual fluorescence measurements were made in the emission mode with the excitation and emission slits set at 10 nm.Emission was monitored near 400 nm, as indicated, and excitation at either 240 or 300 nm.Tryptophan (1.74×10^{-6} M) was used as an external standard to calibrate the fluorescence emission before each spectrum was recorded. These fluorescence intensities were not corrected for detector response and spectral characteristics are presented as directly observed. Excitation spectra of the isolated photoproducts (only) required no such correction, since they were obtained using a Spex Fluorolog fluorimeter which automatically compensates for the changing intensity of the incident beam with wavelength.

A mass spectrum for one of the photoproducts isolated from irradiated poly(dA) was obtained on a Neimeg instrument operating in the desorption chemical ionization mode, as a courtesy of the Department of Chemistry of Columbia University.

Irradiation. Solutions of oligo- and polynucleotides (10^{-4} M residue concentration) in H_2O or in the indicated buffer were irradiated at room temperature with 254 nm light using an apparatus that has been described (6). Nucleosides in 30 % acetone (3×10^{-4} M) were irradiated at room temperature

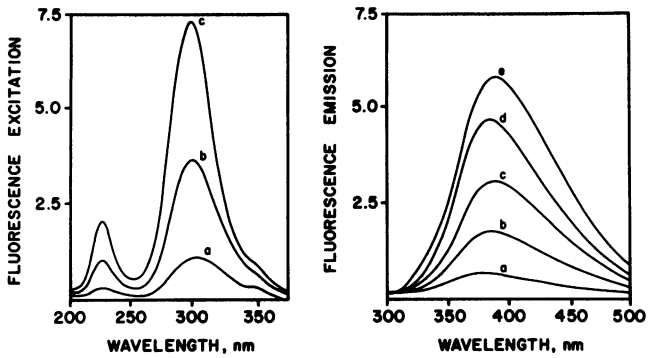


Figure 2. Excitation (left) and emission (right) spectra developed upon irradiation at 254 nm of 1.00 x 10^{-4} M (residues) dApdA in H_2O. Minutes of irradiation: a) 25, b) 50, c) 80, d) 100 and e) 150.

in a Rayonet reactor equipped with 313 nm lamps (Southern New England Ultra-Violet Co., Middletown, Ct.). The quartz reaction vessel was fitted with a Pyrex sleeve to eliminate wavelengths less than 280 nm.

RESULTS

Fluorescence characteristics of purine dehydrodimer formation

Irradiation of an equimolar mixture of 8-BrAdo and Ado at 313 nm in 30 % acetone-70 % water leads to the development of excitation bands at 226 nm and 325 nm with fluorescence emission at 400 nm (all such values are uncorrected unless otherwise specified) (Figure 1). The fluorescence properties of the photoproduct formed by this reaction are very similar to those for 8,8-purine dehydrodimers formed from pairs of ribonucleosides of several bases by similar irradiation treatment and characterized by X-ray crystallography (2). Hence, these fluorescence properties are a signature of the 8,8-purine dehydrodimer moiety.

Irradiation at 254 nm of dApdA in water also leads to the development of very similar fluorescence excitation (226 and 300 nm) and emission (390 nm) (Figure 2). Fluorescence excitation and emission at these wavelengths is also induced, though much less efficiently, upon irradiation at 313 nm of poly(dA), pdApdA, dApdA, or a mixture of 8-BrAMP and AMP in 30% acetone (data not shown).

Irradiation at 254 nm of other oligonucleotides with purine nearest neighbors, dGpdA and dGpdG, also gives rise to fluorescence emission, although to a lesser extent than in those containing adenine neighbors. There is a

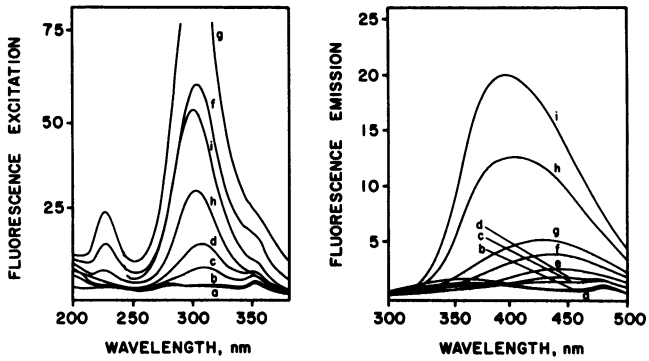


Figure 3. Excitation (left) and emission (right) spectra developed upon irradiation at 254 nm of 1.21 x 10^{-4} M (residues) poly(dA) in the pH 7.2 buffer. Minutes of irradiation: a) 0, b) 20, c) 82, d) 145, e) 247, f) 342, g) 550, h) 1200 and i) 1500. Note that the excitation spectra at 1200 and 1500 min (spectra (h) and (i)) were recorded at 0.1 x the scale sensitivity used at the other times; spectrum (e) is not shown.

very broad emission from overlapping bands in the solution of irradiated dGpdA, with one peak centered about 415 nm, which is the value observed for the 8,8-(ribo)Ado-Gua dehydrodimer (2). The other peak, centered at 380 nm, could arise from the simultaneous formation of some structural or conformational isomer of the dAdo-dGua photoproduct. In the irradiated solution of dGpdG, there is a single peak with a maximum at 400 nm, within 5 nm of the maximum for the 8,8-(ribo)Gua dehydrodimer (2).

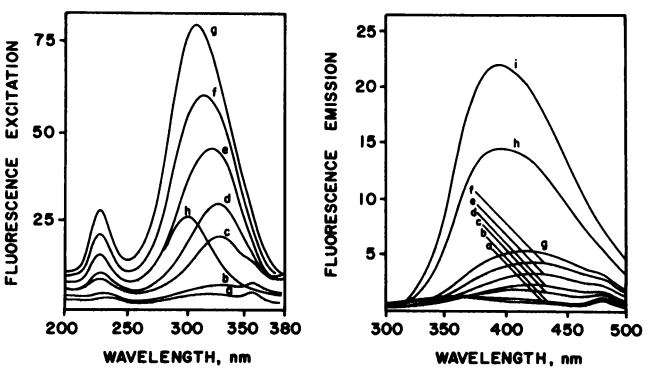


Figure 4. Excitation (left) and emission (right) spectra developed upon irradiation at 254 nm of 1.11 x 10^{-4} M (residues) poly(dA·dT) in the pH 7.2 buffer. Minutes of irradiation: same as Figure 3. Note that the excitation spectrum at 1200 min was recorded at 0.1 x the scale sensitivity used at other times.

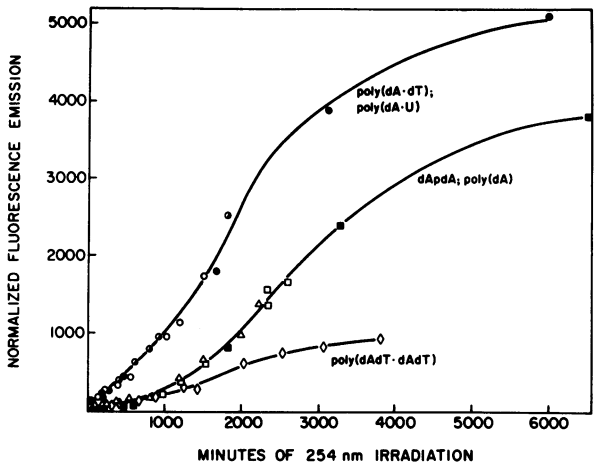


Figure 5. Kinetics of photoproduct formation in the pH 7.2 buffer, monitored by fluorescence emission at 400 nm. The actual fluorescence emission measured for solutions differing in their adenine content was normalized for adenine residue concentration. ●,○ poly(dA•dT), duplicate experiments; ◐ poly(dA•U); △ poly(dA); □, ■ dApdA, duplicate experiments; ◆ poly(dAdT•dTdA).

Conformational dependence of purine dehydrodimer formation

Irradiation of poly(dA) at 254 nm is accompanied by the development of fluorescence excitation (227 nm and 303 nm) and emission (starting at 440 nm and shifting to 400 nm). These fluorescence properties are similar to those described above for the acetone-sensitized formation of the 8,8-Ado dehydrodimer (see Figure 3). When the sugar of the adenine residue is ribose instead of deoxyribose, the fluorescence excitation and emission characteristics after irradiation are very similar (excitation maxima at 242 and 308 nm for both ApA and poly(A); emission maxima at 395 nm for both ApA and poly(A) throughout the course of irradiation). There is, however, a markedly slower rate of photoproduct formation in these cases relative to their deoxy counterparts (14 x for ApA and 7 x for poly(A)) .

In the double helical structure of poly(dA•dT), the development of this fluorescence is promoted (Figure 4). In this case, since the adenine residues absorb only about half of the incident radiation, while the fluorescence emission yield is about the same, it is apparent that the efficiency of photoproduct formation is much greater in the double helix than in poly(dA). This difference in yield is more apparent in the kinetic plots in Figure 5, where fluorescence emission is normalized for adenine residue concentration. This enhanced fluorescence from the double helix has the same spectral

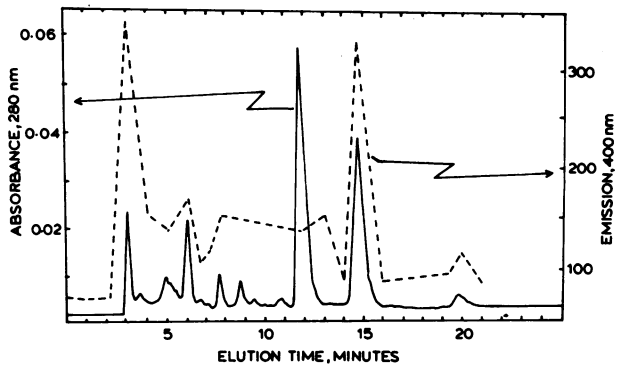


Figure 6. Ion exchange HPLC of pdApdA (3.7 ml, 50 μg/ml) irradiated at 254 nm for 260 min. 2.5 μg in 50 μl d.H_2O were applied to a SAX column and eluted at 1 ml/min using the following potassium phosphate (pH 3.3) gradient: 0.110 M for 2.5 min, 0.110 M → 0.153 M over the next 15 min. The elution profile was continuously monitored by measuring A_{280}, and later, fluorescence emission spectra were recorded on selected fractions. The peak at 11.7 min contains the starting material. The peak at 14.7 min contains the Porschke photoproduct, which appears first and gradually accumulates as irradiation proceeds. The peak at 3 min, a photoproduct that appears later, is not retarded by the column, so it is presumed to contain a dehydrodimer moiety that has lost its internucleotide phosphate.

dispersion as does the fluorescence of irradiated poly(dA), suggesting that the enhancement cannot be due to formation of the 6-4 thymine dimer photoproduct in the poly(dT) strand of the helix, the fluorescence of which has somewhat different spectral dispersion (7). Moreover, fluorescence emission due to the 6-4 thymine dimer photoproduct does not account for the observed exponential rise in fluorescence emission.

Indeed, the plots in Figure 5 strongly suggest that the rate at which the emission increases is significantly influenced by the conformational features of the species being irradiated. The double helical arrangements in poly(dA•dT) (B DNA-type helix) and in poly(dA•U) (A RNA-type helix) appear to promote dehydrodimer formation relative to dApdA and single-stranded poly(dA), both of which have partially stacked, dynamically fluctuating helically twisted base residues; and as noted above, the rates are substantially slower yet for ApA and poly(A) (not shown), whose base stacking overlap may be even less favorable for C8 - C8 reactivity. On the other hand, the alternating adenine-thymine sequence in double helical B DNA-type poly(dAdT•dTdA) seems to inhibit the development of fluorescence. In this case there are no adenine nearest neighbors in the helix, and in fact, the fluorescence properties (excitation at 230 nm and 350 nm, emission at 430 nm) resemble more closely

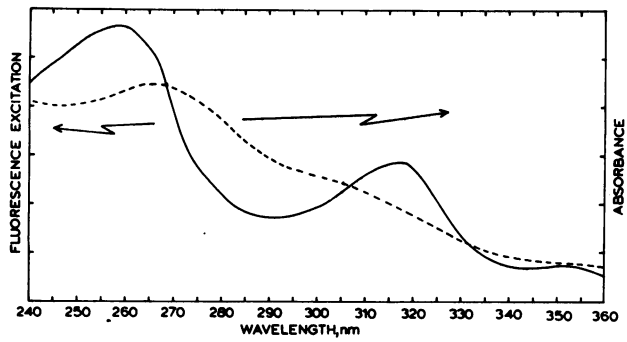


Figure 7. True (corrected) excitation spectrum and absorption spectrum for the peak fraction eluted at 14.7 min from the chromatogram shown in Figure 6. Emission was monitored at 390 nm.

those for the fluorescent hydrolysis product formed from dTpdA, dTpdApdT, dTpdApdTpdA, and poly(dAdT · dTdA) irradiated at 254 nm (8). Hence, the fluorescence developed with poly(dAdT · dTdA) represents the kinetics of formation of a photoproduct different from that formed in the other four cases shown in Figure 5.

Identity of the Porschke photoproduct

As shown above, the fluorescence emission near 400 nm of irradiated oligo- and polyadenylate samples can serve as a sensitive indicator of 8,8-adenine dehydrodimer formation. In the case of irradiated pdApdA, the peak eluted from the ion exchange column at 14.7 min (Figure 6) showed a fluorescence emission λ_{max} very near 390 nm (not shown, but cf. Figure 2). The true (corrected) excitation spectrum and the absorption spectrum of this fraction at pH 3.3 are shown in Figure 7. It is evident that these two spectra are compatible. However, it will be noticed that the excitation spectrum differs significantly from the uncorrected excitation spectrum that develops in the course of photoproduct formation (Figure 2). While both excitation spectra have peaks with λ_{max} near 320 nm, the lower wavelength peak falls near 260 nm for the isolated photoproduct, but closer to 230 nm for the photoreaction mixture. The primary reason for this difference must be that in the photoreaction mixture, most of the energy near 260 nm is absorbed by the unreacted adenine residues.

When the pH of the solution containing the isolated photoproduct is raised to 7, the absorption spectrum changes significantly to one with a broad peak with λ_{max} very close to 260 nm and a broad shoulder above 280 nm that very gradually tapers off with increasing wavelength. This spectrum at pH 7

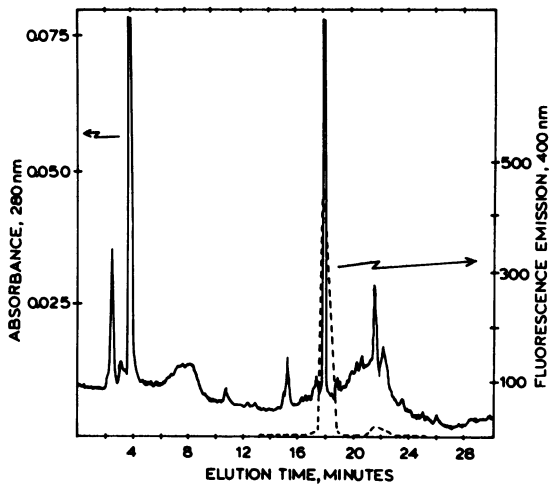


Figure 8. Reversed phase HPLC of an enzymatic hydrolysate of poly(dA) irradiated at 254 nm for 300 min. 50 µl of the hydrolysate (50 µg/ml) were applied to an ODS column which was then eluted at 1 ml/min using the following methanol/20 mM potassium phosphate-pH 3.3 gradient steps: 0 % MeOH/100 % KH_2PO_4 for 5 min; 0 % MeOH/100 % KH_2PO_4 → 35 % MeOH/65 % KH_2PO_4 over 10 min; a 5 min plateau at this composition; 35 % MeOH/65 % KH_2PO_4 → 70 % MeOH/30 % KH_2PO_4 over 10 min; a 10 min plateau at this composition. The column was then returned to the initial solvent composition over 5 min and allowed to equilibrate for another hour prior to reuse. The elution profile was monitored continuously by measuring A_{280}, and later, fluorescence emission spectra were recorded on selected fractions. The peak at 3.5 min, which was not retained by the column, contains undigested poly(dA) and that at 14.9 min contains dAdo, its limit digest product. The peak at 18 min contains the major digest photoproduct, while that at 22 min contains some dehydrodimer moiety that has lost its internucleotide phosphate.

(not shown) is essentially the same as that reported by Porschke for his photoproduct (1). This spectral similarity at pH 7 together with the close correspondence of the fluorescence excitation spectra of the 8,8-Ado dehydrodimer and of the isolated pdApdA photoproduct lead to the conclusion that these substances all have the same 8,8-Ado dehydrodimer moiety.

Enzymatic hydrolysis of irradiated poly(dA) yielded one prominent and one minor fluorescent peak eluted from a reversed phase HPLC column at 18 and 22 min (Figure 8). Their elution positions indicate that these photoproducts are less polar than unmodified dAdo, which elutes near 15 min. The true fluorescence excitation spectra for these poly(dA)-derived photoproducts (Figure 9) differ slightly, i.e., λ_{max} is 290 nm for fraction 18 and 304 nm for fraction 22; but they both differ markedly from the excitation maxima for the pdApdA (Porschke) photoproduct near 260 and 320 nm. These differences may

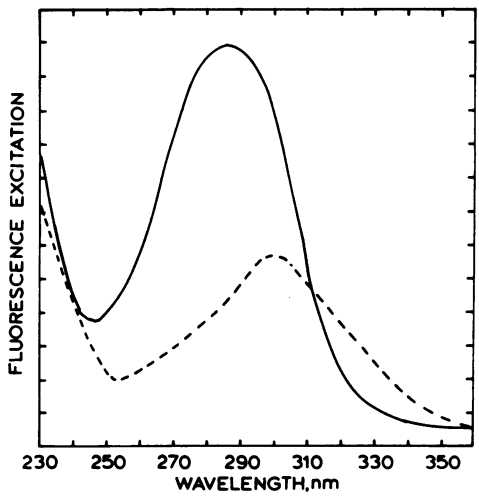


Figure 9. True excitation spectra for fractions eluted at 18 min (solid line) and 22 min (dashed line) from the chromatogram shown in Figure 8. Emission was monitored at 390 nm.

originate in several ways. While the Porschke photoproduct is a highly constrained structure due to the dimeric linkage between the adenine residues and the phosphodiester bond linking their backbone, the latter bond has been enzymatically digested in the products isolated from irradiated poly(dA), giving the linked adenine moieties freedom to rotate. In addition, some of the strain of dimerized bases may be relieved by photomediated phosphodiester or glycosyl bond breakage (9,10). The difference between the two photoproducts isolated from irradiated poly(dA) may be due to the less polar one having lost one of its sugar moieties as well as the inter-residue phosphate.

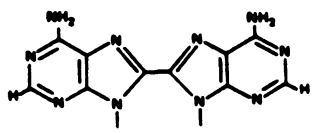
Fraction 18 showed a peak at 502 (m/e) in its mass spectrum, which correlates with the molecular weight for a dimeric deoxyadenosine species that has lost two protons, i.e. the dehydrodimer.

DISCUSSION

The method of photochemical synthesis together with the structure determined by X-ray analysis of the 8,8-guanosine-purine nucleoside dehydrodimers demonstrates the reactivity of 8-purinyl radicals towards any other purine moiety. This allows us to attribute the similar fluorescence excitation and emission that develops in the course of 254 nm irradiation of oligo- and polynucleotides containing purine nearest neighbor residues to the

formation of 8,8-purine dehydrodimer residues. The corrected excitation spectra for the isolated poly(dA) and dpAdpA photoproducts and the mass spectrum of the former support this interpretation. Furthermore, benzimidazole, a purine analog, also forms a dehydrodimer when exposed to 254 nm light (11).

From the structure of the 8,8-adenine dehydrodimer moiety



Formula 1

it is apparent that the extended aromaticity would give rise to the type of fluorescence excitation and emission characteristics that have been observed in the present work. Such a structure would seem to have two predictable consequences, each of which is evident in the foregoing results. One is that the C8-C8 linkage will introduce local strain into the oligo- or polynucleotide backbone, which may result in breakage of a glycosyl bond of the dehydrodimer or a phosphodiester bond associated with it. Such breakage would account for the shift of the fluorescence emission to shorter wavelengths during prolonged irradiation (see Figure 3), and possibly also for the two related photoproducts isolated from irradiated poly(dA) with slightly different spectral characteristics (see Figure 9). The second consequence is that with the glycosyl and phosphodiester bonds intact, the close contact of the linked purine rings will partially quench the inherent fluorescence of the "free" dehydrodimer. Breakage of a glycosyl or phosphodiester bond would eliminate this close contact, and this probably accounts for the exponential rise in fluorescence development evident in Figure 5.

The adenine residue photoproduct described by Porschke (1) was originally characterized in terms of slightly attenuated ultraviolet absorption and significantly altered circular dichroism of the irradiated species. By exploiting its highly specific fluorescence characteristics, we have now identified this purine residue photoproduct as the 8,8-dehydrodimer. Although the excitation spectrum of this photoproduct differs somewhat from those for the two photoproducts enzymatically liberated from irradiated poly(dA), the emission spectra of all three photoproducts are quite similar. This is consistent with their dimerized base moieties being the same. In that case, as suggested above, the differences in the excitation spectra for these

photoproducts could be due to differences in the covalent attachment of the two base moieties of the dimer to the ribosephosphate chain elements.

The 313 nm acetone-sensitized formation of the dehydrodimer in dApdA suggests the possibility that purine dehydrodimers may occur *in vivo* by photosensitization involving endogenous ultraviolet absorbing moieties, similar to the para-aminobenzoic acid-sensitized formation of thymine dimers by longer wavelength ultraviolet light (12), as well as by sunlight. It has been widely assumed that ultraviolet-induced adenine lesions must be biologically insignificant because quantum yield measurements have suggested that they occur as much as ten times less frequently than pyrimidine cyclobutane dimer lesions (1). However, the basis for estimating such photoproduct formation is ambiguous; and a low frequency type of 6-4 pyrimidine dimer has recently been suggested as being the biologically more significant pyrimidine dimer lesion (13). It is also known that when the cyclobutane-type pyrimidine lesions are efficiently repaired, other base lesions remain (14). A purine dehydrodimer could exert a biological effect by blocking DNA replication or by inducing an error-prone repair process (15). The discovery of a damage-specific DNA-binding protein that can recognize unknown ultraviolet-induced adenine lesions (16) makes it particularly likely that the 8,8-adenine dehydrodimer described in this report is a biologically relevant lesion of consequence.

ACKNOWLEDGEMENTS

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