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Photoinduced Electron Transfer, Fluorescence, and Intrastrand Migration of Reactive Intermediates in Pyrenyl Sensitizer - Modified DNA Duplexes

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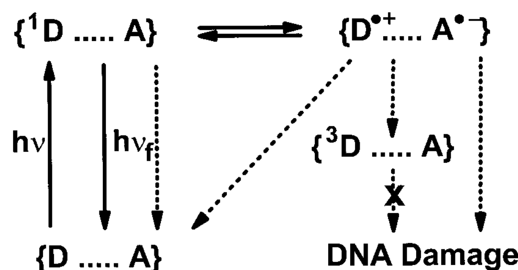
Introduction

The characteristics of electron transfer reactions between photoexcited polynuclear aromatic sensitizers and neighboring bases in DNA complexes are of great importance for understanding the mechanisms of Type I photodynamic reactions that lead to DNA damage.[1] The photosensitizer fluorescence decay pathway competes with electron transfer and other photochemical reactions and is therefore a useful tool for studying the characteristics of these non-radiative phenomena. The radical ion pair formed by the initial electron separation can decay non-radiatively to the triplet excited states and to the ground state of the photosensitizer-DNA complexes. The radical ions can also undergo a variety of chemical reactions that ultimately culminate in DNA damage, e.g. strand cleavage. The essential steps are summarized in the following simplified scheme (non-radiative processes are indicated by dotted lines):



Scheme I

Depending on the redox potentials, either the photosensitizer molecule or a DNA base can act as the electron donor (D) or the electron acceptor (A). In this scheme, it is arbitrarily assumed that D is photoexcited (the multiplicities of the singlet and triplet excited states are denoted by the usual superscripts). The D and A species may be either covalently linked, or form noncovalent complexes {D ..... A } with one another so that diffusional processes can be neglected.

During the course of our structure-activity studies of adducts derived from the binding of highly reactive metabolites of the polynuclear aromatic compound benzo[*a*]pyrene to DNA[2], we noted that the fluorescence of the pyrenyl residues is quenched by nearly two or more orders of magnitude. The low fluorescence yields of the adducts were attributed to photoinduced electron transfer phenomena[3-7] which can also give rise to direct (or frank) DNA strand breaks with low quantum yields. Using site-specifically modified oligonucleotide duplexes in which the site and base-sequence context of the modified base bearing the covalently attached photosensitizer are completely specified, we have shown that direct strand cleavage can occur up to seven bases away from the site of photoexcitation.[8] These observations indicate that reactive intermediates can migrate from base to base in double-stranded DNA. Recently, metallointercalators[9,10] covalently

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From the Executive Director

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In science, perhaps more than the rest of society, change is constant. In Center laboratories, Mike Rodgers, who was setting up picosecond experiments when he came to Bowling Green in the late 1980s, now does transient experiments with femtosecond systems. Alex Mejiritski, who came to our labs with a background in surface science, helped us establish atomic force microscopy so we now routinely look at surface dimensions of a few nanometers. Scientists don't fear change. On the contrary, most spend their waking hours trying to figure out ways to incorporate the latest techniques productively in their research programs.

I never really thought about how much laboratory chemistry has changed from my student day to the present time until I heard the Governor of Ohio speak at a recent meeting of economic development professionals in our region. On that pre-election occasion, the Governor was congratulating anyone in the audience who would listen, and himself mainly, for retaining 5,000 low-paying jobs for our area. When he talked about which educational institutions promoted this retention, the Governor singled out two-year colleges in our area. Four-year colleges and universities were never mentioned. The Governor's position clearly seems that education should prepare an individual for the first job, the cheaper the better. After all, that's the job market his policies are creating.

For the last several years, in spite of economic prosperity in the United States, our politicians have offered plenty of rhetoric indicating Ohio's university system, like many others, costs too much. While we have no argument that universities could be more cost effective, a university education is still one of the best investments a person makes in a lifetime. My question is why have we had such a hard time recently in getting our message out?

Clearly, some people in society need to be trained to meet specific needs of the job market. In these instances training institutes have an important role. But if a college education is doing what it should, the degree recipient will be prepared for lifelong learning and for the inevitable changes society will force on each graduate throughout the course of a working life. Thomas Edison, literally adored around the United States as one of the most inventive entrepreneurs in American history, had more losing ideas than winners. The recording of sound is an excellent example. Though Edison invented the phonograph and started the first recording company, in his opinion, the only legitimate way to record sound was on a wax cylinder. The only voices worth hearing were those that had no vibrato. So Edison's recording company systematically sterilized vocal sound by removing any wobble while recording a singer's voice. When Victor discovered that sound could be recorded on a flat disc, invented the windup Victrola and marketed discs of opera singers that, for that day, were as near as possible to the authentic thing, Edison's son, who ran the Edison record company according to the principles of his domineering father, was soon out of business.

Had our Governor been in charge of the education agenda in Edison's day, it would have been easy and good for business in the short term to encourage young people to study to repair Victrolas or learn the essentials of wax cylinder manufacturing. But these young people would have been anything but well served, and therein lies the danger of some current political attitudes. When the Victrola or the typewriter becomes obsolete, so do the people expert in their repair or operation. Unless the educational system follows the example of change and productivity modeled by the scientific community, many other low-paying jobs in our region may be gone long before the Governor has an opportunity to address those economic professionals again.

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tethered to single nucleotides in oligodeoxyribonucleotide duplexes were found to sensitize hot alkali-labile sites in duplex DNA distant from the locations of the photosensitizers. These effects were attributed to the migration of holes through the DNA π-stack.[9] The base-to-base migration of photoinduced oxidative damage has also been observed in unmodified oligodeoxynucleotides using 193 nm excimer laser pulse excitation and transient absorption techniques.[11] The recent surge of interest in electron transfer phenomena in photosensitizer-DNA complexes[6,7,9-12] has been recently reviewed by Netzel.[13,14]

The redox reactions of excited photosensitizer molecules with DNA and the formation and fates of radical ion pair intermediates are of particular interest for understanding the mechanisms of sensitized photooxidative DNA damage. In this article we summarize the results of our recent studies on photoinduced electron transfer and the photosensitized, site-specific DNA strand cleavage experiments using the benzo[*a*]pyrene metabolite-nucleic acid model systems that we have developed.

The Benzo[a]pyrene Diol Epoxide-DNA Adduct Model Systems

The interactions of the mutagenic and carcinogenic metabolites of polycyclic aromatic hydrocarbon (PAH) compounds[15,16] such as benzo[*a*]pyrene (B[*a*]P) with DNA are of great interest for studies of structure-biological activity relationships in chemical carcinogenesis.[2] In living cells, B[a]P is metabolized by P450 mixed function oxidases to a variety of oxygenated derivatives including phenols, ketones, and epoxides. Because all of these metabolites are more soluble in aqueous solutions than B[a]P itself, their excretion is facilitated. The biologically most important metabolites of B[a]P are the stereoisomeric bay region diol epoxides 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydroB[a]P (*anti*-BPDE, or simply BPDE, Figure 1), in which the epoxide and the 7-OH groups are on opposite sides of the plane of the pyrenyl aromatic ring system (Py). The (+)-7*R*,8*S*,9*S*,10*R* BPDE enantiomer binds more extensively to native DNA than the (-)-7*S*,8*R*,9*R*,10S enantiomer and is thus biologically more important.[15]

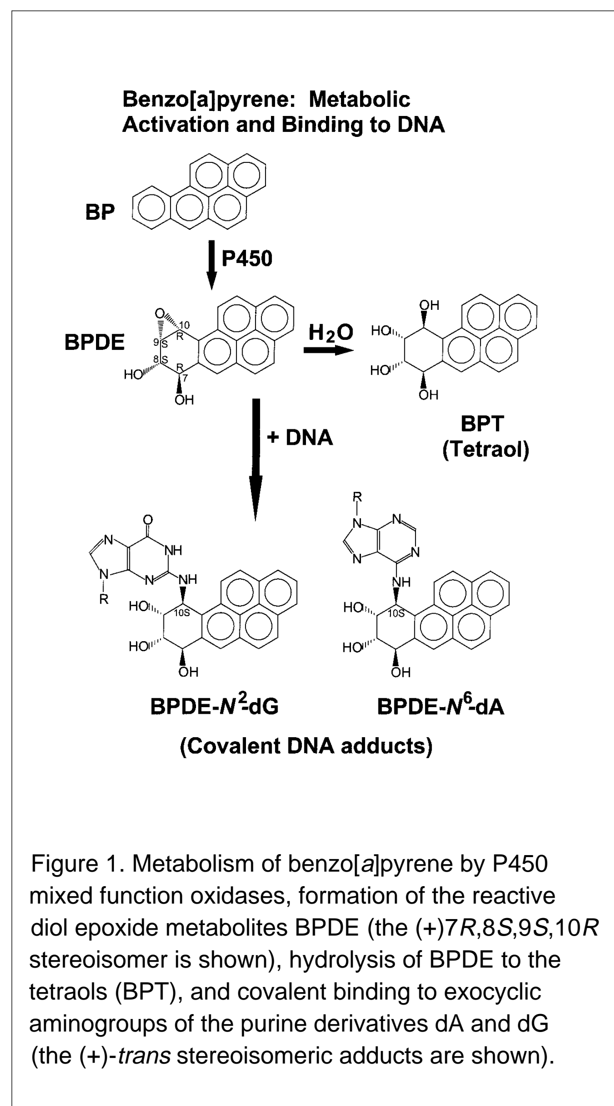

Figure 1. Metabolism of benzo[*a*]pyrene by P450 mixed function oxidases, formation of the reactive diol epoxide metabolites BPDE (the (+)7*R*,8*S*,9*S*,10*R* stereoisomer is shown), hydrolysis of BPDE to the tetraols (BPT), and covalent binding to exocyclic aminogroups of the purine derivatives dA and dG (the (+)-*trans* stereoisomeric adducts are shown).

In aqueous solutions, BPDE reacts either with water to form the non-toxic tetraol hydrolysis products 7,8,9,10-tetrahydroxy-tetrahydrobenzo[*a*]pyrene (BPT), or with the exocyclic amino groups of the guanine (N^2-dG) or adenine (N^6-dA) residues in DNA to form covalent adducts (Figure 1). High resolution NMR studies have revealed that the BPDE-N^2-dG adducts embedded in double-stranded oligonucleotides can assume either base-displaced intercalative conformations[17] (addition of N^2-dG with *cis* stereochemistry to the C10 position of BPDE), or minor groove conformations[18] (*trans*-adduct formation), as shown in Figure 2.

The characteristics of photoinduced electron transfer between the aromatic Py residues and the nucleic acid bases (nucleobases) in aqueous solutions were investigated using three different experimental model systems:

1. Noncovalently interacting electron donor-acceptor systems BPT + dG, dA, dT, or dC .
2. Covalent BPDE-N^2-dG and BPDE-N^6-dA mononucleoside adducts.
3. Covalent BPDE-N^2-dG or BPDE-N^6-dA lesions incorporated into oligodeoxynucleotides of defined base sequence.

In the first model system, BPT forms hydrophobic ground state complexes with the 2'-deoxynucleosides and is subsequently photoexcited; in addition, free BPT molecules are first

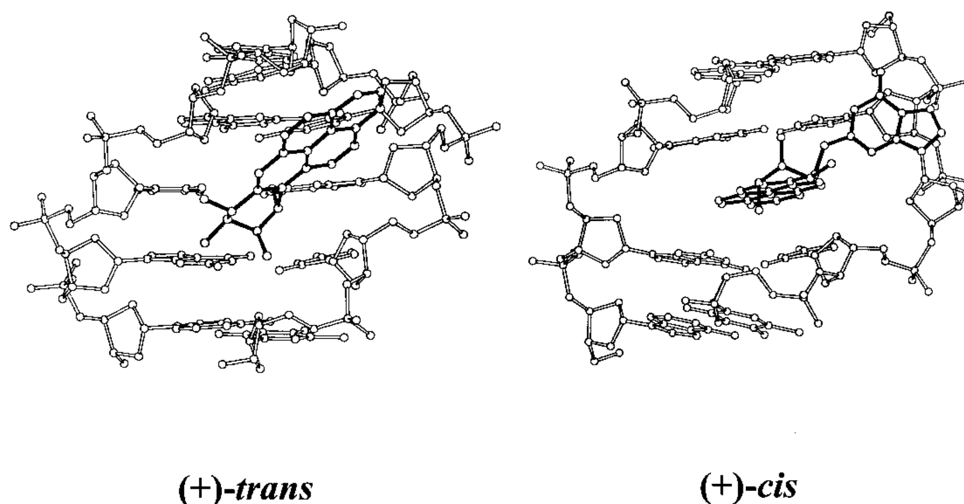

Figure 2. Conformations of (+)-*trans*-BPDE-*N*^{2}-dG adduct[18] (minor groove) and the (+)-*cis*-BPDE-*N*^{2}-dG adduct[17] (base-displaced intercalative conformation) in the 11-mer duplex 5'- d(CCATC[G*]CTACC)•d(GGTAGCGATGG). In the base-displaced intercalative adduct conformers, the hydrophobic pyrenyl residues are inserted between adjacent, intact Watson-Crick base pairs, and the modified dG and the partner dC bases are displaced into the minor or major grooves, respectively. In the minor groove (+)-*trans*-adduct, the hydrophobic pyrenyl residue is positioned in the minor groove, on the 5'-side of [G*]. Reprinted with permission from *Chem. Res. Toxicol.* ***1997***, *10*, 111-146. Copyright 1997 American Chemical Society.

photoexcited and then form diffusive encounter complexes with the 2'-deoxynucleosides. In the second system, the BPDE residues and dG or dA residues are covalently linked to one another (Figure 1). In the third model system, these BPDE-mononucleoside complexes are incorporated into oligodeoxynucleotides in which the photoexcited Py in their singlet excited states, ^{1}Py, can interact noncovalently with neighboring DNA bases as well. Studies of adducts derived from the covalent binding of BPDE to either dC or dT were impractical because of low reaction yields.

Photoinduced Electron Transfer and Fluorescence Yields

The differences in the fluorescence quenching efficiencies of the nucleobases dA and dG are illustrated by the fluorescence lifetimes of the covalent BPDE-*N*^{2}-dG and BPDE-*N*^{6}-dA mononucleoside adducts with 10*S* (+)-*trans*-adduct stereochemistry (Table 1). The fluorescence of the Py residue in the BPDE-*N*^{2}-dG adducts is quenched by a factor > 100, while in BPDE-*N*^{6}-dA adducts the extent of quenching is only ~10%. Consistent with this picture, dG is a strong quencher of the fluorescence of BPT via a noncovalent interaction mechanism, while dA is not.[19] Furthermore, dC and dT are also very efficient quenchers[6,19] since either dC or dT residues flanking BPDE-*N*^{6}-dA adducts strongly quench the fluorescence of the Py residue in the trimers (Table 1). Thus, a covalent linkage between the Py and nucleobases is not critical to the fluorescence quenching process. In aqueous solutions, BPT forms noncovalent ground state association complexes with dG (and with dA) with association constants in the range of ~ 60-200 M^{-1}; the formation of such hydrophobic complexes with the pyrimidines dT and dC is weaker (association constants 10-60 M^{-1}). Both static and dynamic mechanisms play a role in the quenching of the fluorescence of BPT by dG, dC and dT.[6,19] These results parallel those obtained with pyrene[20] and with a pyrenyl residue covalently tethered to oligonucleotides with different flanking bases.[21]

Identification of Py as the Electron Donor or Electron Acceptor by Transient Absorption Spectroscopy

The spectroscopic properties of the Py residues in BPT and BPDE-*N*^{2}-dG adducts are essentially the same as those of pyrene. The transient absorption spectra of pyrenyl radical cations ($Py^{\bullet +}$) and radical anions ($Py^{\bullet +}$) are well established and exhibit maxima at 455-460 and 500-505 nm, respectively. Therefore, the direction of electron transfer in the covalent BPDE-*N*^{2}-dG or noncovalent BPT...dN complexes (dN = dG, dC, or dT) can be established by observing either $Py^{\bullet +}$ or $Py^{\bullet -}$ transient absorption spectra after photoexcitation and subsequent electron transfer (scheme I).

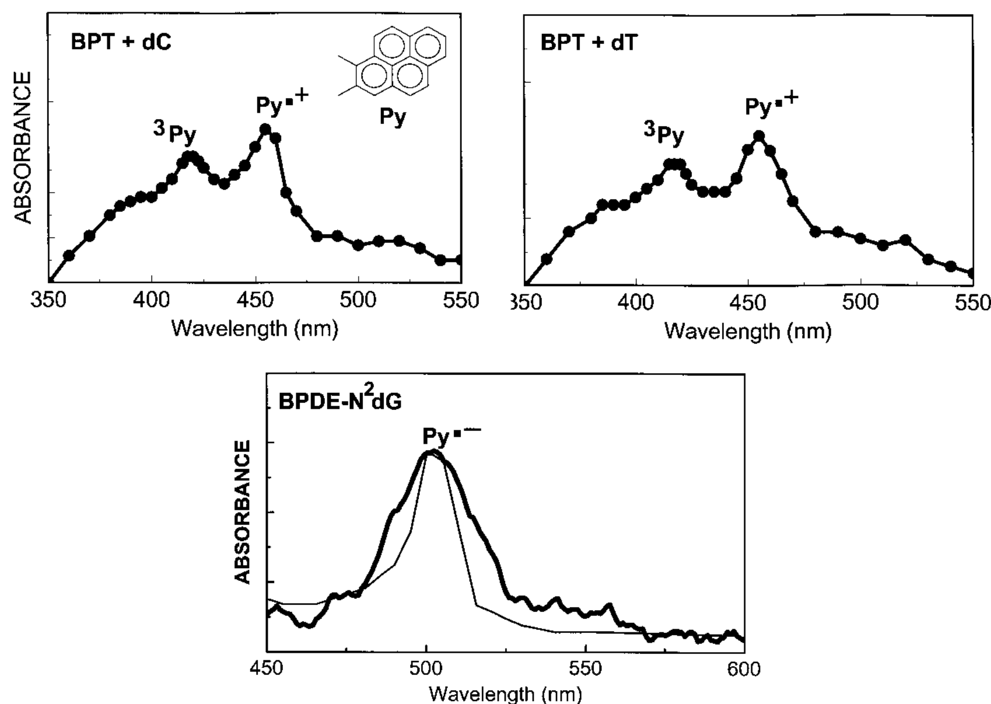

Figure 3. Transient absorption spectra following laser pulse excitation of BPT (delay time Δt = 10 ns) in solutions of 100 mM dC or dT in 20 mM sodium phosphate buffer solution, pH 7 (upper panels),[6] and of covalent BPDE-dG mononucleoside adducts (Δt = 30 ps) in DMF (bottom panel);[4] the thinner line in the lower panel is the absorption spectrum of the radical ion $BPT^{\bullet-}$ in DMF solution.[3] Py is the aromatic residue in BPT or in the covalent BPDE-N^2-dG adduct.

In the case of the covalent BPDE-N^2-dG adducts dissolved in oxygen-free N,N-dimethyl formamide (DMF) solution, the appearance of the $Py^{\bullet-}$ residue with a maximum at 500-505 nm is observed with a rate constant of (9.1±0.9)x10s^{-1} (Figure 3), which is consistent with the fluorescence lifetime of the photoexcited ^{1}BPDE-N^2-dG adducts of 0.11 ns.[4] This result indicates that the polycyclic aromatic Py residue in the photoexcited ^{1}BPDE-N^2-dG adducts acts as the electron acceptor, while the guanine residue acts as the electron donor. In contrast to dG, the nucleosides dT and dC do not quench the fluorescence of ^{1}BPT in polar organic solvents such as DMF or dimethylsulfoxide (DMSO), although all three 2'-deoxynucleosides are excellent quenchers of ^{1}BPT in aqueous solutions. Following laser photoexcitation of BPT in aqueous solutions containing dC (or dT), transient absorption bands with maxima at ~ 455 nm due to the radical cations, $Py^{\bullet+}$, are clearly observable (Figure 3). This result indicates that the Py residue in ^{1}BPT acts as the electron donor. In both cases, transient absorption bands with maxima at ~ 420 nm due to triplet excited states, ^{3}Py, are also observed. The triplet excited states are not sufficiently energetic to undergo electron transfer with the DNA bases and thus cannot give rise to DNA damage (scheme 1). In the case of the quenching of the fluorescence of ^{1}BPT by dG, only the ^{3}Py state, but neither $Py^{\bullet+}$ nor $Py^{\bullet-}$, is observed in aqueous solution (time resolution ≥ 10 ns). However, in solutions of the polar organic solvents,[6] $Py^{\bullet-}$ radical ions are observed. These differences are attributed to a more efficient recombination of the radical ion pair in water than in polar organic solvents. The efficient recombination in aqueous solutions is attributed to the hydrophobic effect, which tends to maintain the two radical ions in close proximity to one another, thus facilitating recombination.[6]

Deuterium Isotope Effect

The fluorescence lifetimes of the ^{1}Py residues in the BPDE-N^2-dG adducts increases from τ = 1.4 ns in H_2O solution to 2.1 ns in D_2O.[6] Solvent isotope effects are also observed in the quenching of the fluorescence of BPT by dG, dC, and dT. In these aqueous solutions, the BPT fluorescence decay profiles can be described in terms of two components: the first component with τ_1 ~ 5 - 6 ns is independent of the nucleoside concentration; the second component, τ_2, decreases

in magnitude with increasing nucleoside concentration. The first lifetime component is attributed to the decay of {^{1}BPT...dN} noncovalent complexes with $\tau_1(D_1O)/\tau_1(H_2O) \sim 1.5 - 2$. In the case of the second component, attributed to the diffusional quenching of the fluorescence of BPT by dT or dC, the ratios $\tau_2(D_2O)/\tau_2(H_2O)$ depend on the concentrations of dN and are in the range of 1.1 - 1.4. These solvent isotope effects suggest that the photoinduced electron transfer is coupled to a proton transfer step[6], as discussed recently in detail.[22]

Redox Potentials and the Direction of Electron Transfer

The thermodynamic driving force for electron transfer, ΔG^o, can be estimated from the Rehm-Weller equation[23] and the relevant redox potentials, E^o:

$$\Delta G^o = e[E^o(dN^{\bullet +}/dN) - E^o(Py/Py^{\bullet -})] - \Delta E_{00} + w$$

where E_{00} = 3.28 eV is the energy of the ^{1}Py singlet excited state, and $w < 0.1$ eV (in highly polar solvents) is the coulombic energy term characterizing the interaction of the two radical ion pairs. The reduction potential of pyrene, $E^o(Pyr/Pyr^{\bullet -})$, is -1.85 V vs. NHE (or -2.09 eV vs. SCE); we assume that the redox potentials for the Py residues are similar to those of pyrene. Thus, nucleobases with reduction potentials of $E^o(dN^{\bullet +}/dN) \approx 1.43$ V (vs. NHE) or less, can be oxidized by the photoexcited ^{1}Py residues. Most recent measurements have shown that E^o for the reduction of 2'-deoxyguanosine (dG) with simultaneous deprotonation of the radical ion is $E_7[dG^\bullet(-H)/dG] = 1.29 \pm 0.03$ V (vs. NHE) at pH 7 in aqueous solution.[24] In polar organic solvents (e.g., acetonitrile) in which deprotonation of $dG^{\bullet +}$ is unlikely, the redox potential of guanine, $E^o(G^{\bullet +}/G)$, is 1.49 V[22] (or 1.58 V[24]) vs. NHE, which is somewhat higher than the effective threshold for the reduction of ^{1}Py, but the quenching of ^{1}Py by dG in DMF is still highly efficient.[2,6] In aqueous solutions at pH 7, the lower value of $E_7[dG^\bullet(-H)/dG] = 1.29$ V suggests that the reductive quenching of the ^{1}Py residues is accompanied by a rapid deprotonation of the $dG^{\bullet +}$ radical cation to yield $dG(-H)^{\bullet +}$, thus accounting for the observed deuterium isotope effect (we assume that E_7, the redox potential at pH 7, for guanine and dG are similar in value). The redox potential of adenine in an aprotic solvent environment is $E^o(A^{\bullet +}/A) = 1.94$[22] (or 2.03 V[24]) vs. NHE; thus, the lack of quenching of ^{1}Py by dA in polar organic solvents can be rationalized. However, in water at pH 7, rapid deprotonation of the radical ion $dA^{\bullet +}$ is predicted with $E_7[dA^\bullet(-H)/dA] \approx 1.42$ V[22,24] vs. NHE, yet dA is not a good quencher of the fluorescence of ^{1}Py, indicating that other factors may play an important role in determining the efficiency of fluorescence quenching.

The redox potentials for the oxidation of dC and dT are higher than those of the two purines.[22] Thus, a quenching mechanism of the fluorescence by a reductive electron transfer to ^{1}Py from dC or dT is not likely. Indeed, while dG is an excellent quencher of the fluorescence of BPT in aprotic polar organic solvents, dC and dT are not.[6] However, in aqueous solutions, both dC and dT are highly efficient quenchers of the fluorescence of BPT. Based on the reduction potentials of dC, and dT in aqueous solutions,[22,25] a fluorescence quenching mechanism in which the pyrenyl residues are oxidized and the pyrimidines are reduced, including a rapid protonation of the $dN^{\bullet -}$ electron adducts, has been proposed.[6,21] The protonation step is coupled to the electron transfer step and involves, most likely, a proton transfer from water[25] to $dN^{\bullet -}$. This protonation lowers the free energy by about 0.9 eV which is, apparently, sufficient to drive the overall electron transfer reaction from ^{1}Py to dC or dT in aqueous solutions. The proton-coupled electron transfer mechanism accounts for the observed deuterium isotope effects.[6] In aprotic polar organic solvents such as DMSO, this proton-coupled step cannot occur, and thus the fluorescence of BPT is not quenched by dC or dT.[6]

Photosensitized DNA Strand Cleavage: Intrastrand Migration of Reactive Intermediates

Irradiation of the Py residues in either single- or double-stranded DNA containing single, site-specifically positioned BPDE-modified dG residues leads to frank, or direct strand breaks. Because the DNA bases do not absorb light in the 340 - 360 nm region, the Py residues can be photoexcited exclusively in this wavelength region. This photochemical damage effect can be induced by exciting the Py residue in BPDE-N^2-dG adducts positioned within oligonucleotides of defined base sequence by either of two methods: (1) by a one-photon excitation process with a xenon-mercury lamp, or (2) with 355 nm picosecond laser pulses from a Nd:Yag laser.[8] In the latter case, $Py^{\bullet +}$ radical cations are produced by a two-photon photoionization process[3]. In both cases, similar patterns of DNA strand cleavage are observed (data not shown). However, hours of irradiation time are needed in the case of the xenon-mercury lamp irradiation, and only a few minutes of irradiation time are needed in the case of picosecond laser excitation in order to observe similar levels of DNA damage. An example of the DNA strand cleavage generated by 355 nm laser pulse irradiation in the BPDE-modified oligonucleotide strand

5'-d(CTCCAT[G*]TACACTCT)

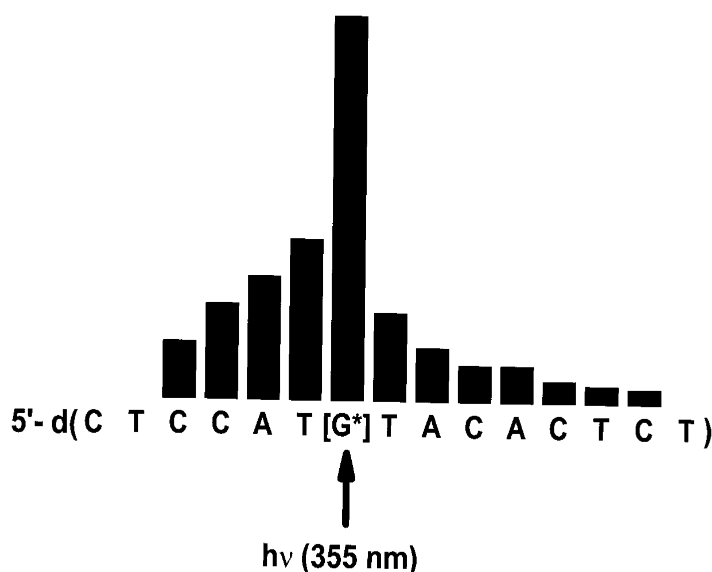

Figure 4. Relative probability of direct strand cleavage at different nucleotide residues following photoexcitation of the Py photosensitizer moiety positioned at [G*] with 355 nm ps laser pulses (fluence: 180 J/cm^2; 10 mJ/cm^2/pulse, ~ 35 ps fwhm). The oligonucleotide shown was complexed with a complementary strand (1 μM duplex concentration) and was dissolved in air-saturated 20 mM sodium phosphate buffer solution during the irradiation.[8]

in a duplex with a complementary strand, adapted from reference 8, is shown in Figure 4. The major direct strand cleavage is observed at [G*], the site of BPDE modification (in this example, about 4% of the strands are cleaved at this site). Strand cleavage is observed, with lower probability, on both the 3'- and the 5'-side of G*; moreover, the probability of cleavage appears to be higher on the 5'-side than on the 3'-side of the BPDE-N^2-dG lesion, and diminishes with increasing distance from G* (Figure 4). Minor strand cleavage events occur also on the complementary, unmodified strand in a non-specific manner, but the probability of cleavage is some 20 times lower at each site than the probability of cleavage at G* on the BPDE-modified strand.

The effects depicted in Figure 4 are oxygen-dependent, since the probability of strand cleavage is reduced if O_2 is removed before irradiation. The overall quantum yield in air-saturated solutions induced by 355 nm picosecond laser photons is of the order of 10^{-4}.

These results clearly indicate that reactive species, resulting from the photoexcitation of G*, can migrate to more distant bases on the modified strand and thus can cause DNA strand cleavage up to 30 Å away from the initially photoexcited G* residue. These mobile, reactive intermediates may be holes as suggested by Barton and coworkers,[9] who observed hot alkali-labile sites at tandem GG bases distant from the sites of attachment of the metallointercalator photosensitizers. Similar kinds of oxidative damage at tandem GG bases have been also observed by Breslin and Schuster using substituted anthraquinone sensitizers as photonucleases.[12] In our experiments, the mobile reactive intermediates, possibly holes,[9] are mostly confined to migrate along the BPDE-modified strand, since the probability of damage within the unmodified strand complementary to the photosensitizer-bearing strand is ~ 20 times smaller.[8] This suggests that the free diffusion of reactive species, e.g. singlet oxygen or free radicals, does not significantly contribute to the site-specific cleavage observed; in that case, strand cleavage would be more evenly distributed among the two strands.

The intrastrand migration of reactive intermediates in double-stranded DNA that cause damage at sites distant from their origin is currently a subject of great interest.[9-12] Our preliminary studies[8] suggest that the BPDE-N^2-dG lesions site-specifically incorporated into oligodeoxynucleotides of defined base sequence are ideal for more detailed mechanistic studies of the migration of mobile reactive intermediates and DNA damage. The conformations of many PAH metabolite-DNA adducts have been established by NMR techniques,[2] e.g. Figure 2, thus specifying the exact positions and conformations of the photosensitizers in the modified DNA duplexes. The details of the initial electron transfer processes that lead to the creation of a radical-ion pair in DNA are beginning to be better understood.[2-7,-9-12,14,21,22] However, the elucidation of the subsequent steps that ultimately lead to DNA strand cleavage at a distance remains an interesting and challenging task. The ultimate resolution of this difficult problem will provide information, at new levels of detail, on the mechanisms by which photosensitizers cause DNA damage by Type I electron transfer mechanisms.[1]

Table. Fluorescence lifetimes and relative yields in 20 mM sodium phosphate buffer solution, pH 7.

Model System	Fluorescence lifetime, τ (ns)	Fluorescence yield (relative to BPT)[d]
7,8,9,10-tetrahydroxytetrahydrobenzo[a]pyrene (BPT)	200±5	1.00
[BPDE]-*N*[2]-dG, [G*][c]	1.5±0.2	0.0075
5'-d(...C[G*]C...), 11-mer duplex minor groove adduct conformation[a] [G*] with 10*S* (+)-*trans* stereochemistry	2.6±0.2[b]	0.013
5'-d(...C[G*]C...), 11-mer duplx base-displaced intercalative conformation[a] [G*] with 10*R* (+)-*cis* stereochemistry	4.0±0.4[b]	0.020
[BPDE]-N[6]-dA, [A*][c]	180±5	0.90
5'-d(C[A*]C), 3-mer[c]	6.4±0.3	0.034
5'-d(T[A*]T), 3-mer[c]	1.5±0.2	0.007

[a]Conformations depicted in Figure 2; [b]weighted mean of 2 lifetimes (relative proportions in parentheses): (+)-*trans:* 1.1 ns (0.81) and 10.0 ns (0.17); (+)-*cis:* 1.5 ns (0.72) and 11.7 ns (0.23). These lifetimes comprise 95-98% of the fluorophores. A minor, long-lived component, attributed to adduct in locally denatured regions of duplex DNA[26] was also observed and not included in the calculation of the mean lifetimes. [c]10*S* (+)-*trans*-adduct stereochemistry. The designation "(+)" means that the adduct was derived from the (+)-BPDE enantiomer. [d]Relative to the fluorescence quantum yield of BPT in aqueous solution.

Acknowledgments

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References

1. Kochevar, I.E.; Dunn, D.A. In *Bioorganic Photochemistry*; Morrison, H., Ed.; Wiley: New York, **1990**; pp 273-315.
2. Geacintov, N.E.; Cosman, M.; Hingerty, B.E.; Amin, S.; Broyde, B.; Patel, D.J. *Chem. Res. Toxicol*. **1997**, *10*, 111-146.
3. Shafirovich, V.Ya.; Levin, P.P.; Kuzmin, V.A.; Thorgeirsson, T.E.; Kliger, D.S.; Geacintov, N.E. *J. Am. Chem. Soc.* **1994**, *116*, 63-72.
4. O'Connor, D.; Shafirovich, V.Y.; Geacintov, N.E. *J. Phys. Chem*. **1994**, *98*, 9831-9838.
5. Shafirovich, V.Ya.; Kuzmin, V.A.; Geacintov, N.E. *Chem.Phys. Lett*. **1994**, *222*, 185-190.
6. Shafirovich, V.Ya.; Courtney, S.H.; Ya, N.; Geacintov, N.E. *J. Am. Chem. Soc.* **1995**, *117*, 4920-4929.
7. Netzel, T.L.; Zhao, M.; Nafisi, K.; Headrick, J.; Sigman, M.S.; Eaton, B. *J. Am. Chem. Soc.* **1995**, *117*, 9119-9128.
8. Li, B.; Mao, B.; Liu, T.-M.; Xu, J.; Dourandin, A.; Amin, S.; Geacintov, N.E. *Chem. Res. Toxicol.* **1995**, *8*, 396-402.
9. Hall, D.B.; Holmlin, R.E.; Barton, J.K. *Nature* **1996**, *382*, 731-735.
10. Hall, D.B.; Barton, J.K. *J. Am. Chem. Soc.* **1997**, *119*, 5045-5046.
11. Melvin, T.; Botchway, T.; Parker, A.W.; O'Neill, P.O. *J. Chem. Soc. Chem. Commun*. **1995**, 653-654.
12. Breslin, D.T.; Schuster, G.B. *J. Am. Chem. Soc.* **1996**, *118*, 2311-2319.
13. Netzel, T. *J. Chem. Educ.* **1997**, *74*, 646-651.
14. Netzel, T. In *Mol. Supramol. Photochem.*; Ramamurthy, V.; Schanze, K.S., Eds.; **1997**, in press.

15. Conney, A.H. *Cancer Res.* **1982**, *42*, 4875-4917.
16. Harvey, R.G. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity,* Cambridge University Press, **1991**.
17. Cosman, M.; de los Santos, C.; Fiala, R.; Hingerty, B.E.; Ibanez, V.; Luna, E.; Harvey, R.G.; Geacintov, N.E.; Broyde, S.; Patel, D. *Biochemistry* **1993**, *32*, 4145-4155.
18. Cosman, M.; de los Santos, C.; Fiala, R.; Hingerty, B.E.; Ibanez, V.; Margulis, L.A.; Live, D.; Geacintov, N.E.; Broyde, S.; Patel, D.J. *Proc. Natl. Acad. Sci. (USA)* **1992**, *89*, 1914-1918.
19. Geacintov, N.E.; Zhao, R.; Kuzmin, V.A.; Kim.; S.K.; Pecora, L.J. *Photochem. Photobiol.* **1993**, *58*, 185-194.
20. Lianos, P.; Georghiou, S. *Photochem. Photobiol*. **1979**, *29*, 13-21.
21. Manoharan, M.; Tivel, K.L.; Zhao, M.; Nafisi, K.; Netzel, T.L. *J. Phys. Chem.* **1995**, *99*, 17461-17472.
22. Seidel, C.A.M.; Schulz, A.; Sauer, M.H.M. *J. Phys. Chem.* **1996**,*100*, 5541-5553.
23. Rehm, D.; Weller, A. *Israel J. Chem.* **1970**, *8*, 259-271.
24. Steenken, S.; Jovanovic, S.V. *J. Am. Chem. Soc.* **1997**, *119*, 617-618.
25. Steenken, S.; Telo, J.P.; Novais, H.M.; Candeias, L.P. *J. Am. Chem. Soc.* **1992**, *114*, 4701-4709.
26. LeBreton, P.R.; Huang, C.-R.; Fernando, H.; Zajc, B.; Lakshman, M.K.; Sayer, J.M.; Jerina, D.M. *Chem. Res. Toxicol.* **1995**, *8*, 338-348.

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Multiple Strategies for UV Tolerance in Cyanobacteria

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Introduction

Cyanobacteria (formerly known as blue-green algae) are photosynthetic members of the domain Bacteria (Eubacteria) that possess two photosystems and evolve O_2 in the same manner as eucaryotic algae and more complex green plants. This is not surprising since it is now well documented that cyanobacteria or their oxygenic ancestors became the chloroplasts of all photosynthetic eucaryotes, an endosymbiotic event or events that undoubtedly took place in the Proterozoic Eon (2.5 - 0.57 x 10^9 years ago). Cyanobacteria as prominent photosynthesizers of this period in the Earth's history (or even earlier), according to most sources, had to learn to tolerate or avoid much higher fluxes of UVB (280-320 nm) than the relatively low intensity that presently reaches the Earth's surface as well as levels of UVA (320-400 nm) comparable to the present. In addition, they were bombarded with UVC (190-280 nm) which at present does not penetrate the Earth's atmosphere. Virtually all UVC and UVB were unattenuated during the Archean and early Proterozoic Eons, before O_2 and ozone accumulated in the stratosphere.[1] At present, under very clear skies at temperate to equatorial latitudes, total UVB may sometimes be as high as 7-8 W m^{-2} and UVA as high as 45-50 W m^{-2}, as compared to values up to ~1100 W m^{-2} for visible wavelengths (~400-700 nm).

Although avoidance of high light and UV radiation (UVR) was a possible strategy, most cyanobacteria are obligately photosynthetic and must have been positioned to receive substantial fluxes of solar radiation, particularly those species which helped to build the extensive, surface accreting stromatolites of the Proterozoic in shallow seas, lakes and springs. Methods for coping with the stress of UV exposure must have evolved early in the history of these microorganisms, genomic responses which may have carried over in part to the chloroplasts of plants.

The influences of UV radiation on microbial populations have been studied with increasing intensity in recent years. One reason is the awareness of decreasing regional ozone levels in the stratosphere that have resulted in an increase of the UVB flux reaching the Earth's surface, and also because microorganisms may show a more immediate and more easily measurable sensitivity to small increases in UV radiation than macrophytes and metazoa.

But as it turns out, cyanobacteria have already evolved various methods (or complex strategies) for coping with both present and possibly higher levels of UV radiation equivalent to levels that occurred in the early Precambrian and which might occur in the future. Since cyanobacteria subsequently invaded a large number of extreme environments (or remained as relicts of similar Precambrian habitats), they must often cope with high solar irradiance in which UV radiation is demonstrably the most inhibitory region of the spectrum. Some cyanobacteria have done so by evolving sunscreen pigments that envelope the cell and work even when cells are at rest or desiccated, by synthesizing internal compounds such as mycosporine-like amino acids (the true value of which is still not well evaluated), by possessing efficient systems for repair of damaged DNA and for replacing UV-damaged compounds, and also by using directed gliding motility for escaping the diurnally high intensities of solar irradiance when soft microbial mats or sediments are present. This abbreviated review considers various effects of UV radiation on cyanobacteria and their compensating responses, with particular emphasis on the reactions that occur or are likely to occur in natural habitats. There is a more extensive recent coverage by Castenholz and Garcia-Pichel.[2]

The Detrimental Effects of UV on Microorganisms

UV radiation may negatively affect molecules of central biological importance and although UV is only a small proportion of the total solar photon flux, the irradiance becomes progressively more detrimental as wavelengths decrease and exponential increases in potential damage to cells might be expected.

The negative effects of UV radiation exposure are many and diverse. At the phenomenon level, photosynthesis, growth, cell differentiation, motility, photoorientation, photomovements, and other processes are affected (see references 2, 3, 4, and 5). UVA (320-400 nm) is primarily associated with production of reactive oxygen species such as $^1O_2$. This form of reactive oxygen causes lipid peroxidation, chlorophyll photobleaching, phycobiliprotein degradation and inhibition of growth, but it also directly affects the D1/D2 protein matrix of PS II[2]. UVB (280-320 nm) results in

some similar damage and inhibits various activities (e.g. RUBISCO, ATP-synthase, synthesis of chlorophyll-a, energy transfer from phycobilisome to chlorophyll, nitrogen fixation, etc.), but it also directly targets DNA and results in dimeric photoproducts between adjacent pyrimidimes and other mutagenic damage, some of which can be corrected by photoreactivation or excision repair (see reference 3).

Cyanobacterial Habitats With High UVR Exposure

Cyanobacteria are conspicuous in many superficial habitats exposed to high solar irradiance, or even shaded habitats that are periodically desiccated. High exposure sites dominated by cyanobacteria include most terrestrial hot spring habitats, intertidal marine flats inundated so infrequently that most eucaryotic competitors and herbivores are absent, and the benthos of shallow hypersaline pools and lagoons. On rocky marine substrates, many cyanobacteria form crusts or small cushions in the high intertidal or supra-tidal zone. Some oligotrophic lakes with severe nitrogen limitation have benthic turfs dominated by cyanobacteria. Unexpected cyanobacterial dominance also occurs in benthic microbial mats in cold water springs, ponds, and lakes, especially in the Antarctic and Arctic.[6,7] A high cyanobacterial biomass develops in these regions probably as a result of a lack of efficient herbivores and ability of these cyanobacteria to withstand freezing which often occurs throughout the water column in some of these regions.

In exposed or shaded terrestrial habitats with periodic or long-term desiccation, cyanobacteria thrive by possessing high desiccation tolerance but presumably also because of a high tolerance to UVR imparted mainly by an extracellular sheath pigment, **scytonemin**, which functions during these periods of metabolic shutdown (more later). The terrestrial habitats with conspicuous cyanobacterial mats or crusts include many of the warm and cold deserts of the Earth where the cyanobacteria may form a soil cover. The harder terrestrial substrates, such as cliff faces, are often covered by periodically wetted, dark cyanobacterial crusts referred to as “tintenstriche” (ink streaks).

The cyanobacteria of the plankton may be exposed, at least periodically, to high solar irradiance when turbulence brings them near the surface under conditions of high light. UVR, however, may penetrate to considerable depth, in some cases UVB to over 30 m and UVA in excess of 60 m (see reference 2). Unicellular cyanobacteria of picoplanktonic size occur abundantly in warm and temperate marine waters, and freshwater types of similar size occur in many lakes, including alpine and subalpine waters. Larger gas vacuolate cyanobacteria in marine waters are represented mainly by species of *Trichodesmium* in warmer waters and in fresh and brackish waters by a number of filamentous and colonial species. These cyanobacteria may simply float to the surface during calm periods with high mortality as a result of exposure to high solar irradiance.

Strategies of UV Tolerance by Cyanobacteria

1. UV Shielding Compounds. UVR absorbing compounds represent a passive mechanism for protection in which a substantial portion of the UV radiation is absorbed and cannot interact with potential cellular targets. The use of sunscreens in microorganisms presents special problems because of small cell size. Since absorption depends on both the concentration of a compound and on the pathlength offered to radiation, the concentrations of absorbing substances required by microorganisms must be high, which requires a significant investment of cell resources. Cell wall or membrane bound sunscreens could be only slightly effective for bacteria; only cells (or colonies) larger than 10 µm can make use of internal sunscreens with low cost/benefit ratios.[8,9] However, many cyanobacteria fall into this larger range of cellular/colonial sizes. In cyanobacteria, an extracellular yellow to brown sheath pigment was observed in some terrestial forms by Nägeli[10] and later named **scytonemin** (Figure 1). This pigment is induced most effectively by exposure to UVA radiation.[11] Scytonemin is a

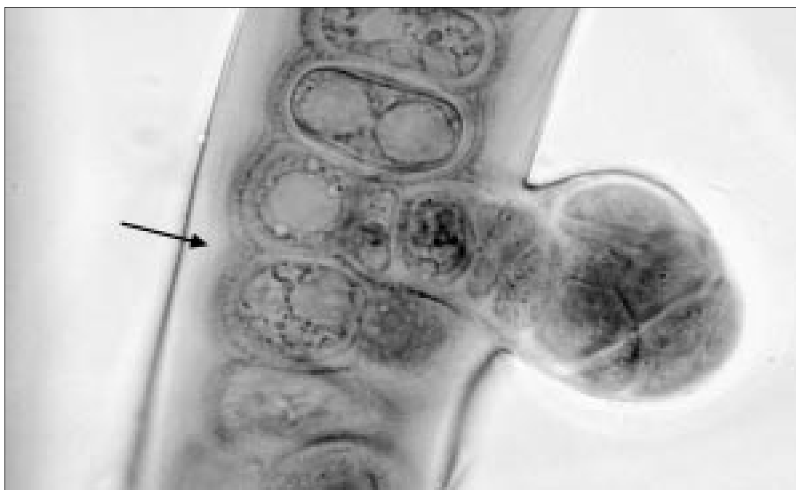

Figure 1. Photomicrograph of a section of branching, filamentous cyanobacterium, *Stigonema* sp., from ultraoligotrophic Waldo Lake, Oregon. Main axis surrounded by a sheath rich in scytonemin (arrow). Width of main axis ~ 50 µm.

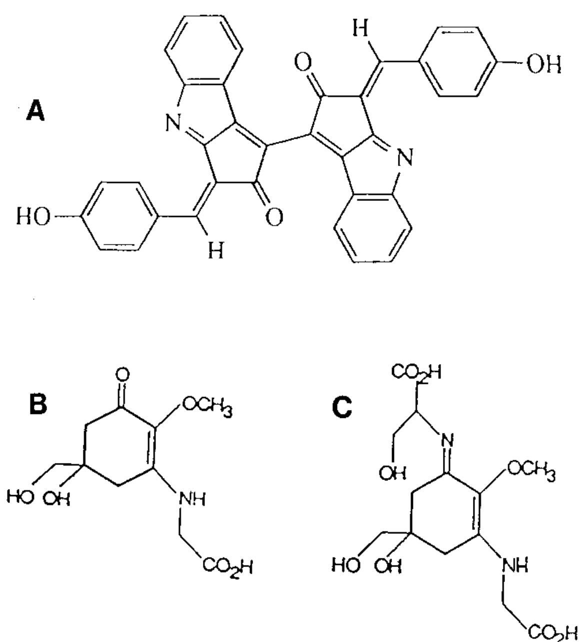

Figure 2. **A**. Structure of scytonemin; m.w. 544 (from reference 12). **B**. The monosubstituted MAA, mycosporine-gly; Abs. max. 310 nm. **C.** The bisubstituted MAA, shinorine; Abs. max. 334 nm.

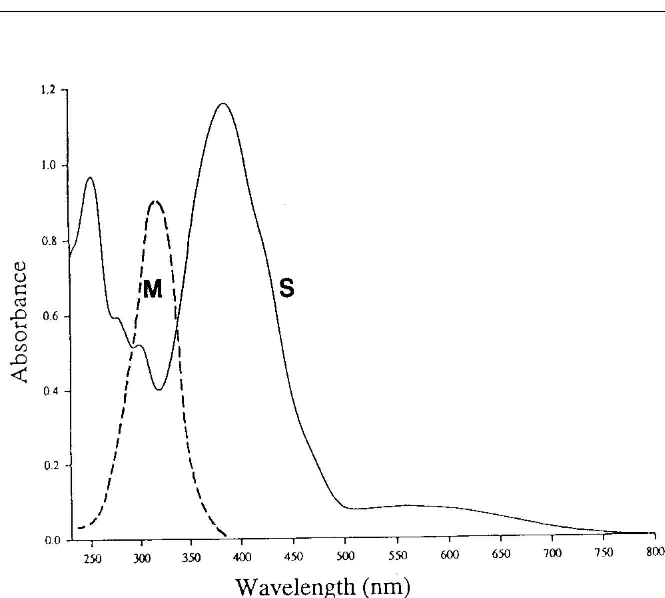

Figure 3. **S.** Absorption spectrum of scytonemin in tetrahydrofuran (from reference 12). **M.** Absorption spectrum of a MAA in 0.2% acetic acid in water; Abs. max. ~ 320 nm.

dimeric indole alkaloid with no closely related compounds known among natural products (Figure 2) and is most probably synthesized from aromatic amino acid residues.[12] The screening by scytonemin is most efficient at the longest wavelengths of the UV (max ~370 nm in vivo), although it absorbs throughout the solar UV range including UVC (Figure 3). Its role as a sunscreen was clearly demonstrated in an isolate of terrestrial *Chlorogloeopsis* sp.,[13] and strong evidence for its role is available from isolates and collected material.[11] It was shown recently that scytonemin is also responsible for UVR screening in a variety of cyanobacterial lichens.[14] Cultures of cyanobacteria may accumulate as much as 5% of the cellular dry weight as scytonemin; field samples may reach an even higher specific content. Scytonemin-producing cyanobacteria are unknown from the planktic environment[11] but are common as benthic or encrusting cyanobacteria of the shallow habitats described above. A frequent common denominator for presence of scytonemin seems to be periodic exposure to high solar irradiance under conditions of restricted metabolism. The high investments required for effective use of scytonemin, as mentioned earlier, may pay off mainly when exposure is linked to periods of metabolic inactivity such as when desiccated. Competition with other cyanobacterial forms that rely on active repair mechanisms would then be successful.

In 1969 Shibata reported that colorless UV-absorbing compounds, which are now known to belong to the family of compounds known as **mycosporine-like amino acid derivatives (MAAs)**, accumulated in large quantities in cyanobacterial cells.[15] This family of substances encompasses a series of colorless, small molecular weight, water soluble compounds which have a single absorption maximum within the UVB/UVA range. Structurally they are condensation derivatives of a cyclohexenone ring and amino acid (or imino alcohol) residues (Figure 2). Their synthesis probably originates from the first part of the shikimate pathway.[16] Monosubstituted MAAs absorb maximally around 310 nm, whereas bisubstituted MAAs absorb maximally at longer wavelengths (between 320 and 360 nm) (Figure 3). They occur in large concentrations in fungi, eucaryotic micro- and macro-algae, and lichens. They also occur in a variety of marine invertebrates, where they are thought to be taken from the diet (see reference 2). In a single survey of 20 cyanobacterial isolates from high insolation environments, 13 contained one or several MAAs.[17] Many cyanobacterial isolates from marine, freshwater, terrestrial or planktonic origin contain MAAs. Although some have been identified, most still await chemical characterization. Shinorine,

asterina-330, porphyra-334 and mycosporine-gly all occur in various cyanobacteria (Figure 2). The MAA in *Nostoc commune* was shown to be extracellular and linked to oligosaccharides in the sheath.[18] These glycosylated MAAs represent the only known example of MAAs that are actively excreted and accumulated extracellularly and therefore allowed to act as a true UV screen.[19] The evidence presented for the primary sunscreen role of intracellular MAAs is inconclusive, and it is possible that they serve more than one role in the cellular metabolism of all or some organisms. The hypothesis that intracellular MAAs serve as sunscreens cannot be discounted, however.[20] In cyanobacteria, a constitutive level of MAA appears to be present under all growth conditions, but the specific content can be significantly raised by exposure to UVA/UVB radiation with a maximum effect at 320 nm.[17] The single-cell sunscreen effect of intracellular MAAs in cyanobacteria is modest (10-30% of incident photons were intercepted in a fairly large-celled *Gloeocapsa* sp.), although colony-formation and mat-type growth may substantially increase the efficiency. The concentration of the sunscreen is relatively low (well below 1% of the dry weight, in most cases), and it seems that the accumulation of intracellular MAA may provide a small benefit for a small investment. There may be physiological limitations to the accumulation of osmotically active compounds such as MAAs within cells.[8] It is possible that the maximal specific content of MAA in the cells is regulated by osmotic mechanisms. This is consistent with the fact that field populations of halotolerant cyanobacteria have unusually high MAA contents.[21]

It is common to find anecdotal allusions in the scientific literature as to the UV protective role of carotenoids. Carotenoid absorption maxima occur mainly in the visible spectral range (i.e. > 400 nm) but often tail into the UVA region (< 400 nm),[22,23] and some have a small absorption peak in the UVB region. However, their function as UV screening pigments would be minimal. In cyanobacteria, it is likely that carotenoids have a largely indirect UV protective function as quenchers of photosensitization products such as triplet chlorophyll, singlet O_2, peroxy radicals, and also as inhibitors of free radical reactions.[24,25] High intensity visible wavelengths (especially blue) and UVA are especially effective in producing the photoproducts which carotenoids have the ability to "quench". During long term exposure to high irradiance, very high ratios of combined carotenoids to chlorophyll-a and phycobilins occur usually as a result of a lowering of the contents of the latter two pigments. Cyanobacterial cells with high carotenoid to chlorophyll/phycobilin ratios are better able to tolerate higher light intensities (and probably UVR too), particularly at suboptimal growth temperatures when cell metabolism is slowed.[26]

2. Avoidance responses by motile cyanobacteria. There are numerous reports of the downward movement of motile "oscillatorian" cyanobacteria from microbial mat surfaces into the mat matrix or into soft sediments during periods of high solar irradiance (Figure 4). Motility by *gliding* occurs in most filamentous cyanobacteria. Although the downward movements usually amount to only about 1 mm or less, the attenuation of visible and UV radiation within this short distance is extreme.[27] Vertical movements of this type were previously described in shallow hot or warm spring mats, in shallow subtidal, intertidal marine and hypersaline mats or sediments, and in some freshwater mud flats (see reference 2). Although there is a positive correlation between increasing irradiance and downward positioning, there are not many quantitative data available. Garcia-Pichel et al.[28] found that the distance of downward descent (up to about 0.5 mm) in soft microbial mats of hypersaline salinas of Guerrero Negro, Mexico, correlated well with the increase in surface irradiance, and that the fluence rate of visible irradiance at the depth of the main cyanobacterial band during the daylight hours (except for early morning and evening) corresponded to that of photosynthetic saturation and not of photoinhibition (Figure 4). Thus, it is possible that optimization of photosynthetic irradiance was achieved and not simply the avoidance of harmful intensities. In a laboratory measurement of vertical movement in another mat type, UVB was particularly effective as a cue.[29] In a more recent outdoor study of two species of motile cyanobacteria in the hypersaline mats of Guerrerro Negro, it was shown that the downward and upward movements were most sensitive to the natural levels of UVR (C. Kruschel and R.W. Castenholz, unpublished). That is, upward movements in bright daylight were effectively prevented by UVB or UVA in contrast to bright visible irradiance alone. Downward movements, beginning from low light, occurred primarily in response to increasing intensities of UVA and visible irradiance. The spectral/irradiance responses appear quite complex and apparently depend on the species involved and the mat type (C. Kruschel and R.W. Castenholz, unpublished). In the same study, it was also shown that natural UVB and UVA radiation are the spectral regions most inhibitory to photosynthesis in the predominant motile cyanobacteria. It is likely that vertical positioning of motile cyanobacteria within a mat is a reflection of primary avoidance of surface intensities higher than ~ 1-2 W m^{-2} of UVA and ~0.1-0.2 W m^{-2} of UVB (C. Kruschel and R.W. Castenholz, unpublished). The avoidance of high solar irradiance by migration appears to be a strategy that results in tolerance of habitats exposed to high UVR and visible radiation without the problem of long term direct exposure and the need for physiological acclimation. Indeed in the few cases investigated, the generally high cell

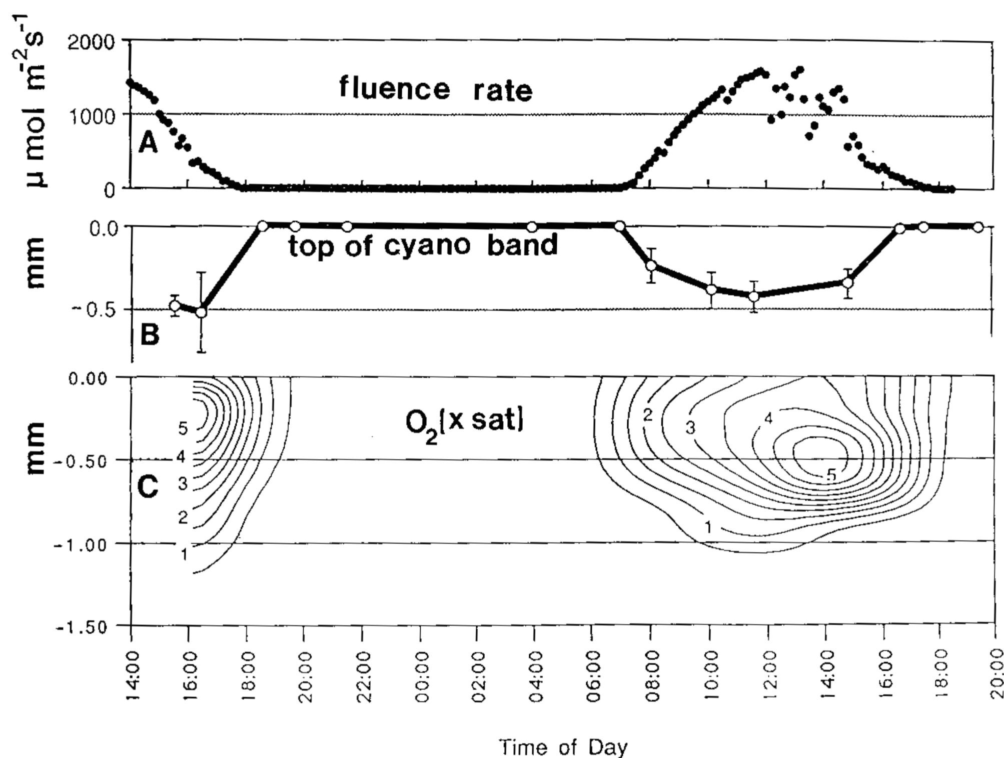

Figure 4. Dynamics of a migrating cyanobacterial band (consisting of mainly of *Oscillatoria* sp. and *Spirulina* sp.) from a microbial mat at Guerrero Negro, Mexico, together with diel fluence rate at surface of mat, and O_2 concentration as times saturation (modified from reference 28).

content of major light-harvesting pigments (phycobilins and chlorophyll-a) was retained, allowing the cells to take advantage of low photon fluence rate such as in early morning, late afternoon, and periods of overcast. High cell contents of photosensitizing pigments, such as chlorophyll and phycobilins, result in severe photodynamic damage if forced to remain under high UV or visible radiation.

3. Tolerance to UV radiation requiring active repair or synthetic processes. If microorganisms are not protected from UVR by avoidance movements, shielding, quenching, or "detoxifying" compounds, it is apparent that repair processes (or rebuilding by synthetic processes) is the last alternative allowing the cyanobacteria to persist in nature under a moderate to high UV flux. The acclimation of cells to UVR may involve active repair mechanisms in which damaged targets are partially substituted or the damage is repaired without the need for *de novo* synthesis of all the components. This is exemplified by the DNA repair mechanisms. Alternatively, increased synthesis of damaged target proteins, or the possible synthesis of UV-resistant forms of target proteins may also be involved in acclimation to UVR. The recurring nighttime period under natural conditions may be required for normal recovery of some of the daytime damage. A dark period has been shown to greatly benefit the replacement of the D1 protein and the recovery of PS II activity damaged under high light intensity.[30] Exposure of DNA to UVB and/or UVC radiation can cause several types of DNA lesions, the most common being the photodimerization of adjacent pyrimidine bases, although adducts and cross-links may also be formed. DNA-repair mechanisms are universal for basically all types of cells. All cyanobacteria studied have some type of excision repair and photoreactivating activity.[31] Photoreactivation appears to be very efficient in comparison with the process in *Escherichia coli*.[5] While there is direct evidence for the presence of dark excision repair in cyanobacteria, they seem to rely heavily on photoreactivation for DNA repair.[31,32]

4. Other cyanobacterial responses to UVR. Radiation in the 295-390 nm range (UVB and UVA), at an intensity that resulted in 30-40% cell survival, changed the pattern of protein synthesis in the unicellular cyanobacterium *Anacystis nidulans* R-2 (= *Synechococcus* PCC 7942).[33] Sixteen "UV-shock" proteins were synthesized. Heat shock without UVR induced the synthesis of seven proteins that were similar, in terms of SDS-PAGE mobility, to seven of the "UV-shock"

proteins. Six distinct proteins with similarity to the "UV-shock" proteins were also induced by oxidative stress. More recently, Shibata et al.[34] demonstrated that a form of pro-oxidant lumazine from cyanobacterial cells generated superoxide anion under "near" UVR. Shibata et al.[33] suggested that exposure to UVR shifted synthetic metabolism to the production of enzymes that scavenge reactive oxygen moieties thereby limiting UVR damage. Superoxide dismutases in the cytosol and thylakoids and catalase-peroxidases, for example, are well known defenses against reactive oxygen species, and their synthesis is often increased under various stress conditions (see reference 2).

Summary and Conclusions

An increasing number of research scientists are considering the effects of UVR on photosynthetic "procaryotes" and microalgae. Many are reductionists and fail to consider the many environmental factors that modify the direct effects of UVR. However, studies have progressed in recent years from simply measuring the effects of UVB or UVA alone in the laboratory with little or no background of visible irradiance to at least trying to simulate natural conditions. In addition to the detrimental effects of UVR and the strategies of tolerance or avoidance exhibited by cyanobacteria and other photosynthetic microorganisms, it is now beginning to be realized that UVR can also be used as a signal or cue for various biochemical or motility responses. In these cases the UV receptors and transduction systems are still unknown.

Many cyanobacterial species combine most of the responses discussed above to counter the detrimental effects of UV irradiance. Most species with scytonemin-bearing sheaths also produce intracellular MAAs. However, not all combinations are possible. For example, cells surrounded by sheaths which bear scytonemin as a screening pigment are essentially immotile and therefore cannot use the directed motility response of avoidance. Some of the migrating species, on the other hand, do not possess scytonemin or MAAs.[17] Many sedentary species do not have sheaths, scytonemin, or MAAs, and it is presumed that most of these depend on repair or rapid synthesis of damaged components. During bright sunny periods when these species show depressed photosynthetic rates with UVR exposure, nighttime rehabilitation may be an absolute requirement for survival of the population in nature. The importance of all the "strategies" discussed earlier have not been fully evaluated in the context of a natural setting and seldom for over long periods (e.g. months). In addition, the complex effects of UVR (or the absence thereof) on whole communities of microorganisms have only begun to be studied (see reference 2).

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References

1. Kasting, J.F.; Holland, H.D.; Kump, L.R. In *The Proterozoic Biosphere, a Multidisciplinary Study*; Schopf, J.W.; Klein, C., Eds.; Cambridge Univ. Press, **1992**, 159.
2. Castenholz, R.W.; Garcia-Pichel, F. In *Ecology of Cyanobacteria; Their Diversity in Time and Space*; Whitton, B.A.; Potts, M., Eds.; Kluwer Acad. Publ.: Dordrecht, **1997**, in press.
3. *Environmental UV Photobiology*; Young, A.R.; Björn, L.O.; Moan, J.; Nultsch, W., Eds.; Plenum Press: New York, **1993**.
4. Häder, D-P.; Watanabe, M.; Furuya, M. *Plant Cell Physiol.* **1986**, *27*, 887.
5. Jagger, J.; *Solar-UV Actions in Living Cells*; Praeger, NY, **1985.**
6. Vincent, W.F.; Castenholz, R.W.; Downes, M.T.; Howard-Williams, C. *J. Phycol.* **1993**, *19*, 745.
7. Tang, E.P.Y.; Tremblay, R.; Vincent, W.F. *J. Phycol.* **1997**, *33*, 171.
8. Garcia-Pichel, F. *Limnol. Oceanogr.* **1994**, *39*, 1704.
9. Garcia-Pichel, F. *Scientia Marina* **1996**, *60*, 73.
10. Nägeli, C. *Neue Denkschrift Allg. Schweiz. Natur Ges.* **1849**, *10*, 1.
11. Garcia-Pichel, F.; Castenholz, R.W. *J. Phycol.* **1991**, *27*, 395.
12. Proteau, P.J.; Gerwick, W.H.; Garcia-Pichel, F.; Castenholz, R.W. *Experientia* **1993**, *49*, 825.
13. Garcia-Pichel, F.; Sherry, N.D.; Castenholz, R.W. *Photochem. Photobiol.* **1992**, *56*, 17.
14. Büdel, B.; Karsten, U.; Garcia-Pichel, F. *Oecologia* **1997**, *112*, 165.
15. Shibata, K. *Plant Cell. Physiol.* **1969**, *10*, 325.
16. Favre-Bonvin, J.; Bernillon, J.; Salin, N.; Arpin, N. *Phytochemistry* **1987**, *26*, 2509.

17. Garcia-Pichel, F.; Castenholz, R.W. *Appl. Environ. Microbiol.* **1993**, *59*, 163.
18. Böhm, G.A.; Pfleidere, W.; Böger, P.; Scherer, S. *J. Biol. Chem.* **1995**, *270*, 8536.
19. Ehling-Schulz, M.; Bilger, W.; Scherer, S. *J. Bacteriol.* **1997**, *179*, 1940.
20. Garcia-Pichel, F.; Wingard, C.E.; Castenholz, R.W. *Appl. Environ. Microbiol.* **1993**, *59*, 170.
21. Oren, A. *Geomicrobiol. J.* **1997**, *14*, 231.
22. Goodwin, T.W. *The Biochemistry of the Carotenoids, Vol. I, Plants*, 2nd ed.; Chapman and Hall: London, **1980**.
23. Koyama, Y.; Hashimoto, H. In *Carotenoids in Photosynthesis*; Young, A.; Britton, G., Eds.; Chapman and Hall: London, **1993**, 327.
24. Krinsky, N.I. *Pure Appl. Chem.* **1979**, *51*, 649.
25. Britton, G. *FASEB J.* **1995**, *9*, 1551.
26. Castenholz, R.W. In *Taxonomy and Biology of Blue-green Algae*; Desikachary, T.V., Ed.; University of Madras, India, **1972**, 406.
27. Garcia-Pichel, F.; Bebout, B.M. *Mar. Ecol. Progr. Series* **1996**, *131*, 257.
28. Garcia-Pichel, F.; Mechling, M.; Castenholz, R.W. *Appl. Environ. Microbiol.* **1994**, *60*, 1500.
29. Bebout, B.M.; Garcia-Pichel, F. *Appl. Environ. Microbiol*. **1995**, *61*, 4215.
30. Constant, S.; Ohad, I.; Kirilovsky, D. In *IX International Symposium on Phototrophic Prokaryotes, Abstracts*; Peschek, G.A.; Löffelhardt, W.; Scmetterer, G. Eds.; University of Vienna, **1997**, 143.
31. Levine, E.; Thiel, T. *J. Bacteriol.* **1987**, *169*, 3988.
32. Blakefield, M.K.; Harris, D.O. *Photochem. Photobiol*. **1994**, *59*, 204.
33. Shibata, H.; Katsuya, B.; Ochiai, H. *Plant Cell Physiol*. **1991**, *32*, 771.
34. Shibata, H.; Noda, T.; Ogura, Y.; Suginaga, K.; Matsui, Y.; Ozoe, Y.; Sawa, Y.; Kona, Y. *Biochim. Biophys. Acta*. **1996**, *1274*, 129.

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