Absorption Spectrum of DNA for Wavelengths Greater than 300 nm

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Although DNA absorption at wavelengths greater than 300 nm is much weaker than that at shorter wavelengths, this absorption seems to be responsible for much of the biological damage caused by solar radiation of wavelengths less than 320 nm. Accurate measurement of the absorption spectrum of DNA above 300 nm is complicated by turbidity characteristic of concentrated solutions of DNA. We have measured the absorption spectra of DNA from calf thymus, Clostridium perfringens, Escherichia coli, Micrococcus luteus, salmon testis, and human placenta using procedures which separate optical density due to true absorption from that due to turbidity. Above 300 nm, the relative absorption of DNA increases as a function of guanine-cytosine content, presumably because the absorption of guanine is much greater than the absorption of adenine at these wavelengths. This result suggests that the photophysical processes which follow absorption of a long-wavelength photon may, on the average, differ from those induced by shorter-wavelength photons. It may also explain the lower quantum yield for the killing of cells by wavelengths above 300 nm compared to that by shorter wavelengths.

INTRODUCTION

Ultraviolet radiation incident on microorganisms can lead to death (1) and mutation (2). Ultraviolet radiation is also harmful to higher organisms; in humans, the deleterious consequences of uv include sunburn, premature aging of the skin, corneal damage, and skin cancer.

Many laboratory studies of the biological effects of uv use 254-nm radiation from low-pressure mercury lamps. In contrast, the spectrum of solar radiation at the surface of the earth contains only wavelengths greater than about 280 nm. In the range from 280 to 320 nm the intensity of solar uv increases rapidly (approximately exponentially) with increasing wavelength, while the sensitivity of most biological targets decreases—also approximately exponentially.

Setlow (3) pointed out that the deleterious biological effect of solar uv should be proportional to an integral of the form

\[
\int \int T(\lambda)\psi(\lambda, t)\sigma(\lambda)d\lambda dt,
\]

where \(\psi(\lambda, t)\) is the flux of solar radiation to which an organism is exposed, \(\sigma\) is the biological sensitivity of the organism as a function of wavelength, i.e., the action
spectrum for the particular effect, and $T(\lambda)$ accounts for shielding of the critical
target. Shielding is the result of the attenuations due to reflections, scattering, and
absorption of the incident radiation. The sensitivity of most biological systems
is much lower for wavelengths greater than 300 nm than for shorter wavelengths (3).
This loss in sensitivity is countered in part by the much higher flux of solar radiation
and the frequently lower absorption losses (higher value of $T$) at these wavelengths.
At a time of maximum insolation, the product $\psi(\lambda)\sigma(\lambda)$ has a maximum value at
about 300 nm (3); thus wavelengths greater than 300 nm play an important role in
the detrimental biological effects of solar radiation.

The primary cellular target for killing and mutations in microorganisms and the
induction of skin cancer in mammals by ultraviolet radiation is thought to be DNA
[see Ref. (4) for a discussion of the data which point to this conclusion]. Although
most data are for the region between 240 and 280 nm, and particularly for 254 nm,
DNA is also usually presumed to be the primary target in the range 290–320 nm.
The absorption spectrum of DNA in the range of 300 to 320 nm and the photo-
physical and photochemical events which occur subsequent to the absorption of
a photon are thus of great interest since collectively they determine the $\sigma$ spectrum.

Measurement of the absorption spectrum of DNA above 300 nm is complicated
by the effects of light scattering, i.e., turbidity. Although photons which are
scattered and not absorbed give rise to apparent optical density, their energy is not
available to form photoproducts. We have used the procedure of Latimer and
Eubanks (5) and also the procedure described by Jagger (6), both of which are
designed to separate the optical density due to true absorption from that due to
scattering. The two deconvolution procedures give essentially identical results.
We report the corrected absorption spectra of DNAs from both procaryotic and
eucaryotic organisms in the wavelength region above 300 nm.

MATERIALS AND METHODS

DNAs from calf thymus, Clostridium perfringens, Escherichia coli B, human
placenta, Micrococcus luteus, and salmon testis were purchased from Sigma
Chemical Company (Saint Louis, MO). In addition, a sample of E. coli DNA was
also obtained from P-L Biochemicals, Inc. (Milwaukee, WI). The DNAs were
purified further by extraction with redistilled phenol followed by extensive dialysis
to remove all traces of the phenol. Samples were then ethanol-precipitated and
redissolved in 10 mM Tris HCl, pH 7.0. Absorption spectra from 300 to 500 nm
were recorded for each DNA using concentrated solutions ($OD_{260} \geq 20$). For
absorption spectra at wavelengths below 300, these concentrated solutions were
diluted in the same buffer so that $OD_{260} = 1$.

The mononucleotides (dAMP, dCMP, dGMP, and TMP) were purchased from
P-L Biochemicals, Inc.

Absorption spectra were recorded with a Cary 118C absorption spectrometer
(Varian Associates, Palo Alto, CA) equipped with a scattered-transmission acces-
sory (model 1862000). Operation of the spectrometer was controlled by a com-
puter (model 4051, Tektronix Inc., Beaverton, OR) via an interface which has
been described elsewhere (7). The computer advanced the wavelength drive of
the spectrometer from one wavelength to the next, typically 1 nm, and averaged absorbance values until the standard deviation of the absorbance was less than 0.001 optical density unit. Spectral arrays were stored on flexible disks for post-experimental deconvolution. Details of the computing system have been described by Sutherland et al. (8).

The wavelength calibration of the spectrometer was checked by determining the apparent wavelength of the lines of a low-pressure mercury lamp and of the deuterium lamp of the instrument. At 250 and 300 nm the instrument calibration was low by about 0.3 and 0.5 nm, respectively, values which are within the tolerances specified by the manufacturer. All spectra were recorded at room temperature (approximately 25°C) using the spectrometer’s quartz–halogen source operated in its high-intensity configuration and with the instrument in the “auto-slit” mode.

THEORY OF MEASUREMENTS

Optical density in a double-beam absorption spectrometer is defined by

\[ D(\lambda) = \log \left( \frac{I_r}{I_s} \right), \]

where \( I_s \) is the intensity reaching the detector after passing through the sample solution and \( I_r \) is the corresponding intensity which reaches the detector through a reference solution consisting of the same buffer minus the component of interest. This definition corrects for reflective losses which occur at the air–cuvette and cuvette–solution interfaces of both the sample and the reference. As used here, \( D(\lambda) \) will also be corrected for slight differences between the sample and reference beams by subtracting the spectrum obtained with reference buffer in both cuvettes.

As defined above, \( D(\lambda) \) is a measure of those photons incident on the sample which do not reach the detector either by virtue of being absorbed or by being deviated in direction (i.e., scattered). The fraction of scattered photons which do not reach the detector is reduced by decreasing the distance between sample and detector and hence increasing the solid angle subtended by the detector. We used two sample positions; in the “normal” position the sample and the detector were separated by about 90 mm, while in the “close” position, the face of the photomultiplier was less than 10 mm from the back of the sample.

As long as the total optical density due to scattering is not too great\(^1\) and the sample is macroscopically homogeneous and nonfluorescent, the optical density can be written as the sum of an absorbance and a scattering term. The absorbance

\(^1\) As the magnitude of the scattering increases so does the probability that multiply scattered photons will reach the detector. These multiply scattered photons travel a greater distance through the sample than do undeviated photons. For wavelengths which are absorbed by the sample, the probability of absorption is proportional to the total path traversed: the magnitude of an absorption band is thus amplified by multiple scattering so that absorption and scattering are no longer separable [see Ref. (9) and references cited therein]. The probability of multiple scattering goes roughly as the square of the fraction of the incident photons which do not reach the detector due to scattering and is thus insignificant so long as only a small fraction of the incident photons are scattered as in the case of the data shown in Fig. 1. Another demonstration that this effect is not significant for the data reported here is that, after correction for the change in concentration upon dilution, the spectra for the concentrated and dilute samples agree in the wavelength region where they overlap.
term is independent of the distance between detector and sample, but the scattering term decreases when the sample is moved from the "normal" to the "close" position since some photons are deviated through an angle great enough to cause them to miss the detector in the former but not in the latter case. Thus we can write expressions for the optical density observed at the two sample positions in the form:

\[ D_n(X) = A(X) + S_n(X) \]  \hspace{1cm} (3a) \\
and \\
\[ D_c(X) = A(X) + \alpha S_n(X), \]  \hspace{1cm} (3b)

where \( D_n \) and \( D_c \) are the optical densities measured with the sample in the normal and close positions, respectively, \( A(\lambda) \) is the true absorbance, \( S_n \) and \( S_c \) are the contributions of scattering for the two sample positions, and \( \alpha = S_c/S_n \).

Latimer and Eubanks (5) provided theoretical arguments and experimental data which indicate that \( \alpha \) is independent of wavelength even though \( S_c \) and \( S_n \) are not. Thus \( \alpha \), which depends on the geometry of the experiment, can be determined from the ratio \( D_c/D_n \) at some wavelength for which the absorbance is zero. Functionally, we calculate the ratio \( D_c(\lambda)/D_n(\lambda) \) for all wavelengths and examine the long-wavelength region to determine the wavelength range over which the ratio is constant (typically 400 to 500 nm). The average value of the ratio in this wavelength range is chosen as \( \alpha \). From Eqs. (3a) and (3b) we obtain

\[ A(\lambda) = \left[ D_c(\lambda) - \alpha D_n(\lambda) \right]/[1 - \alpha]. \]  \hspace{1cm} (4)

The deconvolution procedure is illustrated in Figs. 1 and 2 for a sample of calf thymus DNA. In Fig. 1 we show the optical density spectra for both sample positions. On the right are the optical densities measured at both positions for a concentrated solution (approximately 5 mg/ml); the upper curve is \( D_n \) and the lower curve is \( D_c \). On the left are the spectra for a 100-fold dilution of the concentrated sample; the two spectra are resolved in the plot only near 260 nm, because their difference is small compared to the width of the lines generated by the plotter. In Fig. 2, we show the absorbance spectrum determined by means of Eq. (4).
DNA ABSORPTION SPECTRUM ABOVE 300 nm

Fig. 2. Absorption spectrum of calf thymus DNA plotted on a logarithmic scale. The spectrum was computed from the data in Fig. 1 by the method of Latimer and Eubanks (5). The absorption spectrum of the concentrated sample was divided by 100 and then the absorption spectra of both concentrated and dilute samples were normalized so that the maximum absorbance is equal to 1.0. The spectrum has not been smoothed to provide an indication of the signal-to-noise ratio typically achieved above 320 nm.

plotted on a logarithmic scale. The spectrum of the concentrated sample has been divided by a factor of 100 and the entire spectrum normalized to a value of 1.0 at its maximum. The spectrum of the more dilute sample extends only to 320 nm since at longer wavelengths it becomes too noisy to provide useful data.

The second method we used for deconvoluting the contributions of absorption and scattering involves approximating the scattering contributions by an expression of the form $S = k\lambda^{-n}$ (6). Values of $k$ and $n$ were determined by fitting a straight line to the values of $\log D(\lambda)$ in the long-wavelength region by the method of least squares. Values of $S$ computed from these values of $k$ and $n$ were then subtracted from $D(\lambda)$ for all values of $\lambda$. This procedure was applied separately to optical-density spectra obtained in both the “normal” and “close” positions. Spectra obtained using this procedure were in good agreement with absorption spectra calculated according to the method of Latimer and Eubanks (5).

RESULTS AND DISCUSSION

The relative absorption by DNA for wavelengths greater than 300 nm is a function of base composition. Figure 3 shows the long-wavelength absorption spectra of three naturally occurring DNAs with base compositions ranging from $Cl. perfringens$ with 31% G–C to $M. luteus$ DNA which has 72% G–C. Figure 4 shows the relative absorption at 313 nm for all six DNAs which we studied. The data in Figs. 3 and 4 indicate that there is a reasonable correlation between the fraction of G–C base pairs and the absorption above 300 nm. $E. coli$ may be a special case which will be discussed below.

The shift in the absorption threshold with increasing G–C content follows the absorption spectra of the individual bases. The absorption spectra of the nucleotides dAMP, dCMP, dGMP, and TMP are shown in Fig. 5; the ordinate is logarithmic to facilitate comparisons in the long-wavelength region. The long-wave-
length absorptions of T and C are quite similar. However, G absorbs at slightly longer wavelengths than T and C, while the absorption of A ceases at significantly shorter wavelengths.

The absorption spectrum of double-stranded DNA is determined both by the absorption spectra of the individual components and by interactions between adjacent bases. Below about 290 nm, base–base interactions give rise to hypochromicity of the DNA compared to the sum of the absorption of its individual components. Above 290 nm, the same interactions result in hyperchromicity [see, e.g., Ref. (10) and references cited therein]. The spectra in Fig. 3 and the data in Fig. 4 indicate that absorption by guanine and cytosine (or conversely, the lack of absorption by adenine) plays an especially significant role in the long-wavelength absorption spectrum of double-stranded DNA.

The rapid decrease in the absorption spectrum of calf thymus DNA between 300 and 320 nm continues the trend observed below 300 nm (Fig. 2). Furthermore, the correlation between absorption and G–C content argues that the absorption
observed in this region is characteristic of the DNA being studied and is not due to impurities or methodological artifacts. Above about 320 nm, the decrease in absorption becomes less rapid; a broad shoulder extends past 360 nm. The absorption in this wavelength region is very small, only about one part in 10^4 of the peak absorption near 260 nm. It is possible that some, and perhaps all, of the apparent absorption observed in this region is not intrinsic to the DNA being studied as opposed to contamination by some chromophore which could not be removed by the purifications we employed. Indeed, the sample of *E. coli* DNA obtained from the Sigma Chemical Company (Lot 57C-6830) showed a significantly higher absorbance above 320 nm which was not reduced by repeated extractions compared to the other DNAs we studied. A sample of *E. coli* DNA from P-L Biochemicals, Inc. showed significantly less absorption than the sample from Sigma; spectral properties which we present for *E. coli* DNA are those obtained from the material obtained from P-L. Even so, the 313-nm absorption of this sample is somewhat higher than would be expected on the basis of its G–C content (Fig. 4).

Since all of the DNAs studied (except for *Cl. perfringens* which was the least concentrated sample studied in the long-wavelength region) showed some weak absorption extending to wavelengths greater than 350 nm, we also cannot rule out the possibility that at least part is intrinsic absorption by the DNA.

Spectroscopic and photochemical data suggest that the greater contribution of G compared to A in the long-wavelength absorption of DNA might have significant consequences on subsequent photophysical and photochemical processes. Hydrogen bonding between guanine and cytosine in double-stranded polynucleotides was reported to strongly quench the fluorescence of both bases (11) and the phosphorescence of guanine (12). These results suggest rapid, nonradiative quenching of the excited singlet state of hydrogen-bonded guanine. The quenching of cytosine fluorescence could be due to rapid internal conversion of the cytosine or transfer of the excitation energy to guanine followed by internal conversion. The short lifetime of the excited singlet state of hydrogen-bonded guanine is in contrast to that of adenine since the absorption of a photon by this base at 77 K can result in either exciplex fluorescence or phosphorescence characteristic of the triplet state of thymine [see Ref. (13) and references cited therein]. These spectroscopic
data suggest that photons absorbed by adenine might be more likely to sensitize
the production of pyrimidine photoproducts than photons absorbed by guanine,
although considerable care obviously must be exercised in extrapolating from
77 K spectral data to room-temperature photochemistry.

Setlow et al. (14) reported that the initial rate of thymine dimer formation
resulting from 280-nm radiation at room temperature was greater in the trinucleotide
d(pApTpT) than in d(pGpTpT). Since the trinucleotides were not hydrogen
bonded, the mechanism for this lower reactivity of d(pGpTpT) may differ from that
discussed above. However, the heterogeneous nature of DNA and the differences
between the photophysical processes which occur in guanine compared to adenine
suggest that photons absorbed by guanine or regions of DNA rich in guanine may
be less likely to give rise to pyrimidine photoproducts than photons absorbed by
adenine or adenine-rich regions of DNA. Indeed, Setlow et al. (14) found that the
rate of production for thymine dimers in DNA by 280-nm radiation was greater
by about a factor of 2 in the sequence pApTpT compared to the sequence pGpTpT.
More recently Haseltine et al. (15) showed 254-nm radiation produces more
dimers in DNA in the sequence ATTA than in the sequence ATTG. There is also
the possibility that photons absorbed by guanine might be more likely to result in
cytosine-containing photoproducts; thus the ratio of cytosine-containing photo-
products to thymine-containing photoproducts might increase for irradiation
wavelengths greater than 300 nm.

If the quantum yield for photoproduct formation is lower for regions of DNA
rich in guanine and if the absorption spectrum of DNA above 300 nm is due prefer-
entially to such regions, it follows that the quantum yield for photoproduct
formation would be lower above 300 nm compared to shorter wavelengths. Such
seems to be the case. In Fig. 6, we plot the scatter-corrected absorption spectrum
of human placental DNA and the action spectrum for the killing of stationary-
phase human cells (16). The two spectra are generally similar in shape but not quite
identical, especially above 300 nm. The quantum yield for cell killing is proportional
to the ratio of the action spectrum to the absorption spectrum. Since the magnitude
of the slope of the action spectrum is greater (by about 30%) than that of the
absorption spectrum for wavelengths near 300 nm, it follows that the quantum
yield for lethal photoproduct formation decreases with increasing wavelength.
That is, the biological effect per absorbed photon is greater for wavelengths below
300 nm than for wavelengths greater than 300 nm. At 289 nm, the quantum yield
for cell killing is almost six times greater than at 313 nm.

The scatter-corrected absorption spectrum of E. coli DNA is compared with
action spectra for cell killing [(17, 18), R. B. Webb, personal communication] in
Fig. 7. There is good agreement between the action spectra and the absorption
spectrum in the region between 265 and 300 nm; however, the relative cross sec-
tions for killing at 313 nm and the longer wavelengths are below the absorption
curve. Even if we used the relative absorbance for a DNA with 50% G–C content
interpolated from the data in Fig. 4 (excluding the measured value for E. coli,
which as noted above may be artificially high by as much as 20%) absorption at
313 nm would still exceed the relative action spectrum. Again, the slope of the
action spectrum between 300 and 320 nm is steeper than that for absorption.
The similarity below 300 nm of the absorption spectra of DNA and the action spectra for cell killing shown in Figs. 6 and 7 strongly implicates nucleic acids as the critical molecular target for far-uv damage [cf. Ref. (4)]. However, Figs. 6 and 7 reveal significant differences between action and absorption spectra which can be rationalized in terms of the heterogeneous nature of DNA. Thus the absorption spectrum cannot, as a rule, be substituted in Eq. (1) for the appropriate action spectrum in calculations of the adverse biological effects of solar ultraviolet radiation. For example, the use of absorption spectra in Eq. (1) to estimate the effects of increases in the intensity of short-wavelength solar ultraviolet resulting from decreases in stratospheric ozone would result in a serious underestimate of the expected increase in biological damage as a result of the steeper slope of the action spectrum.

The similarity between the action spectra (240–280 nm) for various biological effects and the absorption spectrum of the DNA of the organism being studied has frequently been cited as evidence that DNA is the molecular target for the particular effect. Action spectra, obtained recently, for killing of mammalian cells growing in culture have shown enhanced sensitivity for wavelengths near 280 nm.
compared to the absorption spectrum (16, 19). In contrast, the absorption spectrum has a relatively higher value above 300 nm than the action spectrum for the killing of nondividing human cells (Figs. 6 and 7). These discrepancies do not establish the significant involvement of other molecules, such as proteins, as targets for cell killing. The heterogeneous nature of DNA and the fact that photons absorbed by different bases may have different probabilities of giving rise to photoproducts can account for the differences observed between absorption and action spectra. Fortunately, there are two independent criteria which can be used to determine if nucleic acids and in particular DNA are the critical biological target for a given effect. First, the action spectrum for the effect should resemble the action spectrum for the photochemical modification of DNA. The best example to date of this reasoning is the demonstration by Rothman and Setlow (19) that the action spectrum for production of thymine-containing pyrimidine dimers in the DNA from Chinese hamster V-79 cells is similar in shape to the action spectrum for loss of colony-forming ability in these cells. The second criterion for identifying molecular targets for uv-induced biological effects is photoreactivation, since photoreactivating enzymes act only on pyrimidine dimers. In all biological systems except higher plants, the ability to enzymatically photoreactivate a biological effect of ultraviolet radiation specifically implicates DNA as the molecular target. In the case of frog cells, Rosenstein and Setlow (20) have shown that the photoreactivable sector is, to within their experimental uncertainty, independent of wavelength between 252 and 313 nm. This result indicates that DNA is the only critical target for this system in the spectral region studied. However, the "oxygen effect" observed for wavelengths greater than or equal to 325 nm (18, 21) indicates that in this spectral region other chromophores may also contribute in vivo.

Fig. 7. Corrected absorption spectrum of E. coli DNA (---) and action spectra for the killing of E. coli 15 T-A-U- (□) (17) and the killing of E. coli WP2s in the presence (○) and absence (○) of oxygen (18). For wavelengths of 313 nm and less, points for survival in the presence and absence of oxygen are coincident (18).
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*Note added in proof:* The deconvolution procedure of Latimer and Eubanks presumes that the optical density due to scattering can be written as a product of a term which is a function of wavelength and another term which is a function of the scattering angle. For scattering materials whose dimensions are not much less than the wavelengths of the radiation being scattered, this condition is, in general, not fulfilled. Thus the procedure of Latimer and Eubanks must be considered an empirical method of separating the effects of scattering and absorption, as is the extrapolation procedure described by Jagger. Care should be exercised in interpreting the results of these deconvolution schemes at the longer wavelengths where the calculated absorptions are very small.

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**REFERENCES**


