Excitation Energy Transfer in DNA: Duplex Melting and Transfer from Normal Bases to 2-Aminopurine[†]

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Received July 14, 1993; Revised Manuscript Received August 27, 1993[®]

ABSTRACT: Absorption and fluorescence excitation and emission spectra of the B DNA duplex decamer d[CTGA(2AP)TTCAG]$_2$, where emission from the 2AP (2-aminopurine) base dominates, have been measured as a function of temperature. A low-temperature excitation band in the 260–270-nm region disappears near the duplex melting temperature, $T_m = 27$ °C, but then reappears at higher temperatures. Singlet–singlet energy transfer thus occurs between the normal DNA bases and the 2AP base in the B-helical conformation and to a lesser extent in the structurally-mobile melted conformation. The measured efficiency of transfer is 4–5% at 4 °C, near 0 at 30 °C, and rises again to 1% at 48 °C. Nearest-neighbor-only singlet transfer is likely. Such transfer does not offer a likely explanation for UV damage distributions in DNA.

Normal deoxyribonucleic acid (DNA)[1] is made up of four bases—adenine, thymine, guanine, and cytosine—whose ultraviolet optical properties are similar but not identical. It is generally difficult to distinguish the contributions of each type of base, for example, to optical absorption spectra of native DNA in the ultraviolet (Gueron et al., 1974). Selective excitation of thymine in synthetic poly(dA-dT)·poly(dA-dT) and poly(dA)·poly(dT) is possible from about 290 to 295 nm (Ge & Georghiou, 1991a,b), but including G or C bases in the polymer prevents analysis of the fate of a photon absorbed by the polymer. If the DNA bases were in fact identical and the polymer structure nearly homogeneous, another possibility must first be considered: an absorbed photon might be considered rather to excite a delocalized excitation in the polymer. Estimates of base–base resonance interaction energies which govern the localization of excitations have given large enough values to suggest that this possibility must be seriously considered, though limitations of the calculation techniques have not been adequate for definitive conclusions (Pearlstein et al., 1976; Gueron et al., 1974). In either case, localized or delocalized excitation, the phenomenon of excitation transfer can occur. In the former case, transfer would be described as excitation "hopping"; in the latter, coherent or wavelike motion can occur in addition to the intrinsic sharing of the excitation among a number of bases.

Absorption of UV light results in photodamage to double- or single-helical DNA which is not random. The formation of the most common UV photoproduct, the thymine–thymine cyclobutane dimer, was observed to occur with probability varying by a factor of up to 30 in the *lacI* gene (Brash & Haseltine, 1982). Brunk showed that thymine photodimers were more likely to form in long stretches of T rather than in short stretches (Brunk, 1973). A study of the distribution of photodimers in UV-irradiated DNA of known sequence

(Gordon & Haseltine, 1982) showed (i) that the steady-state probability of pyrimidine dimer formation depended upon the thymidine (T) content of the site; i.e., TT is more likely to form a dimer than TC, and CT is more likely to form a dimer than CC; and (ii) that the probability of dimer formation depends upon the two flanking bases, but this information is not enough to explain differences in the observed damage. The authors proposed that other, longer-range sequence effects are involved. It has been shown that the overall pyrimidine photodimer probability as well as the site-specific damage probability distribution can be the same for single-stranded as for duplex DNA, so double-helical geometry is not a prerequisite for damage. Rahn has shown the dimer formation probability to be highly temperature dependent for both duplex and single-stranded (denatured) DNA (Patrick & Rahn, 1976). The single-stranded probability decreased linearly with temperature; the duplex probability was constant below the melting temperature, $T_m = 80$ °C, and decreased abruptly at T_m. The probabilities for the two DNAs were equal and linearly decreased above T_m, and the line for the single-stranded data intersected the duplex data again at about 35 °C. Gordon and Haseltine (1982) state that the DNA sequence distribution of dimer formation probability is the same for single-stranded as for duplex, but the temperature was not specified. Another photoproduct, the pyrimidine–pyrimidine 6-4 product, is more likely to form at TC and CC sites than at CT or TT sites in the *lacI* gene of *Escherichia coli* (Brash & Haseltine, 1982). The 6-4 product formation probability was claimed to be higher when the number of A-T base pairs located 5' to the site increased[2] and also when an extended tract of pyrimidines located 5' to the site was present (Wang, 1980). These studies show that UV damage does not simply occur at any base or pair of adjacent bases where light is absorbed. There are three types of mechanisms which can explain this phenomenon. The first assumes that energy deposited at one site in the DNA helix can be transferred to other sites. The rate and direction of energy transfer depend upon the base sequence and/or the structure along the helix. To be of significance,

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[1] Abbreviations: 2AP, 2-aminopurine; A, adenine (6-aminopurine); C, cytosine; dA, deoxyadenylic acid; DNA, deoxyribonucleic acid; dT, thymidylic acid; EDTA, ethylenediaminetetraacetate; G, guanine; poly-(dA-dT)·poly(dA-dT), double-stranded deoxynucleic acid, each strand a polymer of alternating adenine and thymine bases; poly(dA)·poly(dT), double-stranded deoxynucleic acid, one strand a homopolymer of adenine and the other of thymine; T, thymine; tris, tris(hydroxymethyl)-aminomethane; UV, ultraviolet.

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[2] We calculate goodness-to-fit R^2 values for unbiased fits to the 6-4 photoproduct data of Figure 2a of Brash and Haseltine (1982) ranging from $R^2 = 0.05$ for a linear fit to $R^2 = 0.14$ for a second-order polynomial. There appears to us to be no correlation between 6-4 damage probability and the number of A-T base pairs located 5' to the site. The same can be said of the cyclobutane dimer data.

such transfer must occur over distances of more than one or two adjacent bases. The second mechanism assumes that deposited energy does not move but that the probability that a site is damaged depends upon the surrounding structure; e.g., a local defect in the helix may distort the structure of two adjacent bases so as to poise the bases for photochemistry. The third mechanism supposes that a chemically-reactive species is generated from the initial photoexcitation, e.g., singlet oxygen (Sies & Menck, 1992) or free radicals. The reactive species physically diffuses through the solution and damages the more reactive sites on the DNA.

Base-to-base energy transfer, either of single or of triplet excitations, has been suggested as a possible cause of the preferential formation of photoproducts at specific sites in DNA (Setlow & Setlow, 1961; Gueron et al., 1967; Shafra-novskaya et al., 1973; Frank-Kamenetskii & Lazurkin, 1974; Ballini et al., 1976; Suhai, 1984; Rubin & Yegupov, 1987; Georghiou et al., 1990). In this model, excitation energy would tend to accumulate at certain bases due to the variation in base-to-neighboring-base energy-transfer rates and directions along the helix. The weight of the evidence has pointed toward triplet involvement in the formation of cyclobutane dimers but not in the 6-4 photoproduct (Hauswirth & Daniels, 1976; Patrick & Rahn, 1976; Umlas et al., 1985; Rubin & Yegupov, 1987). In the former case, triplet transfer preceded by singlet transfer could not be ruled out. Modeling of this transfer process under the assumption of localization and hopping has been done (Gueron, 1974; Georghiou et al., 1990) but has suffered from a lack of detailed information on electronic properties of the bases in the helix, transfer mechanism, excitation (de)localization, and trapping. The close proximity of adjacent bases, 3–4 Å for stacked bases and 2.5–6 Å for base-paired bases (depending upon how the distance is defined), means that detailed knowledge of their mutual electronic interactions in ground and excited states is crucial to determination of a base-to-base energy-transfer mechanism. To our knowledge, no successful calculation of DNA photo-product damage distribution from energy-transfer rates, either singlet or triplet, has been done. Transfer of UV excitation energy, if it indeed takes place in DNA under *in vivo* conditions, will have an important role in the final disposition of the energy in the DNA helix, be it via delocalization or hopping-type energy transfer. Whether this energy deposition governs the photodimer/photoadduct production remains to be shown.

MATERIALS AND METHODS

The d[CTGA(2AP)TTCAG]$_2$ and d(CTGAATTCAG)$_2$ decamers were prepared as described (McLaughlin et al., 1987). Fluorescence and absorption spectral measurements were performed with samples of duplex concentration 10–11 μM. Solutions contained 0.1 M potassium chloride, 0.1 mM EDTA, and 10 mM Tris-HCl, pH 7.5. Absorption spectra were measured on a Gilford Response II spectrophotometer, using a 1-nm bandwidth. Temperature was controlled by the Gilford thermoelectric sample holder. Data were stored digitally and transferred to a computer for analysis and plotting. Fluorescence spectra were collected using a Perkin-Elmer LF-50 fluorometer, using a Lauda RMS-6 circulator for temperature control. Excitation and emission bandwidths were 2.5 and 5 nm, respectively. Data were again stored, converted to ASCII, and transferred to the computer for plotting. Excitation spectra are corrected for lamp fluctuations and detector wavelength dependence. Fluorescence cuvettes were 0.2 × 1.0 cm, with the excitation beam traversing the

0.2-cm path. The optical densities of the DNA over 0.2 cm at 260 and 307 nm were about 0.4 and 0.02. This higher-than-normal absorbance at 260 nm is required because (i) sample dilution would unacceptably depress the duplex melting temperature and (ii) accurate measurement of the low 307-nm absorbance necessitates a higher absorbance at 260 nm, since measurements at the two wavelengths must be made at the same concentration and long path length cells cannot be used due to sample cost. The relatively high absorbance at 260 nm will cause a reduction of about 30% in the intensity of fluorescence excited at 260 nm, due to attenuation of the excitation beam through the sample. Correction for this reduction of the excitation beam intensity is accomplished by multiplying the measured fluorescence intensity at each excitation wavelength λ by the factor $2.303A(\lambda)/[1-10^{-A(\lambda)}]$, where $A(\lambda)$ = the absorbance at wavelength λ. [Note that $A(307$ nm$)$ = 0.024 where 2AP is directly excited; the corresponding correction factor is about 0.97.] This correction has been done in Figure 3a,b and taken into account in calculating the transfer efficiency. Reabsorption of fluores-cence is not a problem because of the large separation between the emission (370 nm) and the 260-nm absorption and the low value of the 307-nm absorbance.

Melting curves measured by absorption were performed in the Gilford spectrometers using the programmable thermo-electric temperature controller. The samples were placed in stoppered 0.3 × 1 cm path-length cells, heated to about 70 °C to preclude later air bubble formation, and cooled slowly to the lowest temperature in a dry-nitrogen environment, at which point the programmed heating was begun. Heating rates were 1 °C/min. The wavelength was set at 260, 307, or 330 nm, as desired. Melting temperatures were found as follows: (i) from the maximum in the slope of absorbance (260 nm) vs $1/T$; (ii) from computer fits to $A(260)$ vs T with a program generously provided by Doug Turner of the University of Rochester (Frier et al., 1983). Such fits were done by simultaneously fitting the shape and position of the curve, including the slopes of the lines far above and below the melting temperature. Details of such fits will be presented in a forthcoming publication. For present purposes, the melting temperature, T_m = 27 °C, is the only parameter needed.

Energy transfer from a donor molecule to an acceptor can be demonstrated by observing the emission at a wavelength characteristic of the acceptor and scanning the fluorescence excitation spectrum. The transfer efficiency, ϵ_t, defined as the fraction of the excitations absorbed by the donor which result in excitation of the acceptor, can be calculated as follows. Absolute fluorescence quantum yields are normally defined as the ratio of the number of photons emitted by the chromophore to the number of photons absorbed. If one is not concerned with the absolute magnitude of the yield, but rather with relative values or relative changes, the quantum yield of a single chromophore can be written

$$\phi = I(\lambda_{ex})/[1 - 10^{-A(\lambda_{ex})}] \qquad (1)$$

where $I(\lambda_{ex})$ is the fluorescence intensity measured at a wavelength characteristic of that chromophore (often the peak of the emission spectrum) when excited with wavelength λ_{ex} and $A(\lambda_{ex})$ is the sample absorbance measured at wavelength λ_{ex}. Let A_a = the absorbance of the acceptor (2AP) at wavelength λ_{ex}, A_d = the absorbance of the donor (normal DNA bases) at wavelength λ_{ex}, and ϵ_t = the energy-transfer efficiency from donor to acceptor. If two wavelengths can be found such that only the donor absorbs at λ_d and only the

acceptor at λ_a, then the fluorescence intensity when excited at λ_d is

$$I(\lambda_d) = [1 - 10^{-A(\lambda_d)}]\epsilon_t\phi_a$$

$$= \epsilon_t I(\lambda_a)[1 - 10^{-A(\lambda_d)}]/[1 - 10^{-A(\lambda_a)}] \quad (2)$$

where ϕ_a is the intrinsic fluorescence quantum yield of the acceptor (when it is directly excited.) The transfer efficiency is then

$$\epsilon_t = I(\lambda_d)\{[1 - 10^{-A(\lambda_a)}]/[1 - 10^{-A(\lambda_d)}]\} \quad (3)$$

if $I(\lambda_d)$ and $I(\lambda_a)$ are mutually multiplied by the normalization factor which makes $I(\lambda_a) = 1$.

In order to use this equation involving measured relative intensities, it is imperative to ensure that the fluorometer response is identical at the two excitation wavelengths. This will normally be the case with corrected fluorescence excitation spectra in which a single spectral measurement determines both $I(\lambda_d)$ and $I(\lambda_a)$. We choose our two excitation wavelengths at 260 and 307 nm. At 260 nm, the residual absorbance of 2AP could contribute to the fluorescence emission, since excitation would be direct. It will be seen, however, that this residual absorbance is small enough to have a negligible contribution. The requirement for negligible absorbance of normal bases at 307 nm is less strict, as any normal base absorption at 307 nm must be followed by transfer to 2AP before any contribution to fluorescence results. If normal bases absorb 10% of the light at 307 nm, for example, and if the transfer efficiency is 0.05, the contribution of transfer-mediated fluorescence (normal bases → 2AP → emission) will be reduced to 10% × 0.05 = 0.5% compared to the directly-excited 2AP emission. The actual contribution of 2AP to the absorbance at 307 nm was estimated by measuring $A(307$ nm) vs T for both the d(CTGAATTCAG)$_2$ and the d[CTGA-(2AP)TTCAG]$_2$ duplex decamers. The absorbance of the former contains no contribution from 2AP and can be subtracted from the absorbance of the latter to obtain the 2AP absorbance in the d[CTGA(2AP)TTCAG]$_2$ decamer. Since, however, the melting temperature of the d[CTGA-(2AP)TTCAG]$_2$ decamer under the present conditions is 9 °C less than that of the d(CTGAATTCAG)$_2$ decamer [data not shown; see also McLaughlin et al. (1987)], the absorbances of the two decamers were compared at the same point on the melting curve, i.e., when the fraction of single-stranded DNA is the same. This correction to $A(307$ nm) is between –11% and –12% over the entire temperature range 5–50 °C. The overall factor $\{[1 - 10^{-A(\lambda_a)}]/[1 - 10^{-A(\lambda_d)}]\}$ by which $I(\lambda_d)$ must be multiplied to obtain the transfer efficiency (%) is 10.3 ± 0.1 over the entire temperature range. Neglect of the contribution of normal bases to the 307-nm absorbance would add about 1 percentage point to all percent transfer values in Figure 4 and has practically no effect on the temperature dependence of transfer. The excitation wavelengths $\lambda_d = 260$ nm and $\lambda_a = 307$ nm thus satisfactorily fulfill our requirements of nonoverlap as long as the transfer efficiency is not too high.

RESULTS

The UV absorption of the 2-aminopurine-containing DNA decamer is dominated by the absorbance of the normal bases in the 250–280-nm region, since normal bases outnumber 2AP by 9:1 (Figure 1). The absorption of 2AP appears as a small but clear shoulder near 307–315 nm. An absorption spectrum of the (CTGAATTCAG)$_2$ decamer is shown for comparison and to allow estimation of the absorbances of normal and 2AP bases at 260 and at 307 nm. At 260 nm, 2AP contributes
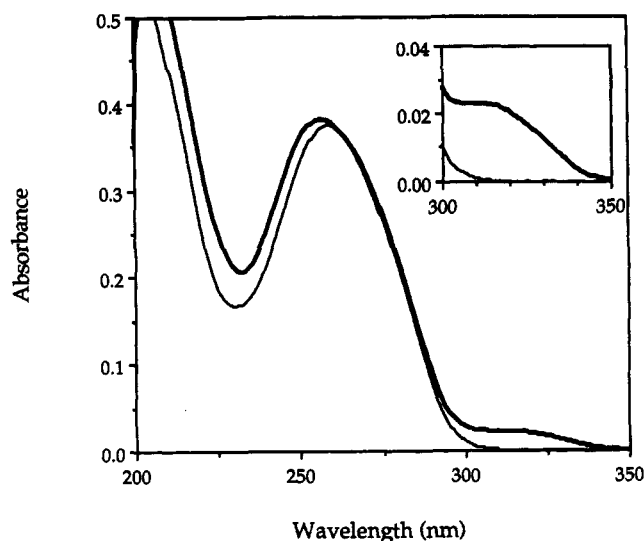

FIGURE 1: Absorption spectra of d[CTGA(2AP)TTCAG]$_2$ (boldface) and d(CTGAATTCAG)$_2$ DNA decamers (lightface). $T = 23$ °C. Inset: enlargement of 300–350-nm region.

about 0.2% of the total absorbance. The fractional absorbance of normal bases at 307 nm is about 0.11, but as noted under Materials and Methods, this has practically no contribution to fluorescence.

The normalized fluorescence excitation spectrum of the DNA decamer [CTGA(2AP)TTCAG]$_2$ shows two changes with temperature: (i) the position of the direct excitation peak of 2AP (near 307 nm) shifts to the blue as the temperature increases, and (ii) a second excitation band in the 250–280-nm region appears at low temperature (Figure 2a). The first of these changes has been reported and attributed to progressive exposure of the 2AP base to water as the helix nears its melting temperature (Evans et al., 1992). The new excitation band is most clearly evident below about 15 °C and shows a peak at 265–270 nm. The observation of a new peak is in itself evidence that the fluorescence excited at 260 nm is not due to residual direct excitation of the 2AP base. This conclusion is confirmed by the small (0.2%) absorbance contribution at 260 nm by 2AP. The magnitude of the new peak relative to that at 307 nm is greatest at the lowest temperature, decreases up to about 30 °C, and increases again slightly from 30 to 48 °C (Figure 2b). The excitation spectrum of the free 2AP base in water, on the other hand, has no peak in the 250–280-nm region and shows no temperature dependence of shape from 4 to 50 °C (Figure 2c).

The fluorescence excitation spectral changes are shown in the differential mode in Figure 3. The spectrum showing the least absorbance at 260 nm, that at 30 °C, has been subtracted from the spectra at each individual temperature, resulting in difference bands with peaks at 265–270 and 325 nm. Again, the free 2AP base spectrum shows a negligible change with temperature (Figure 3c). The 325-nm band results from the blue shift of the main 307-nm band. A pure spectral shift can be shown to contribute little to the 260–280-nm spectrum (data not shown).

The magnitude of the spectral change in the 265–270-nm region can be quantitated by integrating the amplitude of the spectral change shown in Figure 3 from 265 to 270 nm. This temperature-dependent amplitude, multiplied by the almost temperature-independent factor $[1 - 10^{-A(\lambda_a)}]/[1 - 10^{-A(\lambda_d)}]$, is plotted in Figure 4 along with a standard DNA melting curve consisting of the absorbance at 260 nm vs temperature. Figure 4 should be interpreted as curves representing the
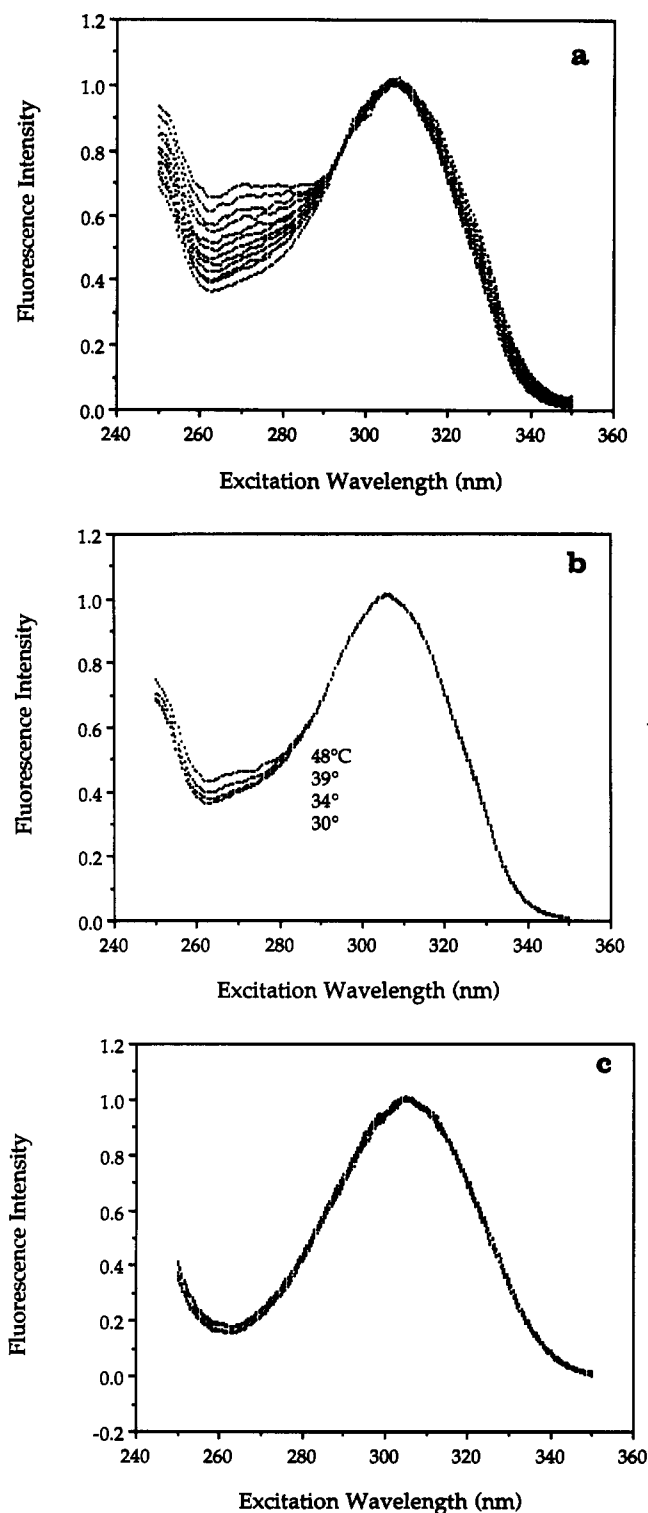
FIGURE 2: Fluorescence excitation spectrum of the d[CTGA(2AP)-TTCAG]₂ DNA decamer as a function of temperature, normalized to a peak amplitude of 1. Emission wavelength 370 nm; excitation bandwidth 2.5 nm; emission bandwidth 5.0 nm. Duplex concentration 10 µM. (a) 4–30 °C, top to bottom at 270 nm. (b) 30–48 °C, bottom to top. (c) Equivalent spectra of 2-aminopurine free base; 10 spectra; 4–50 °C.

general decrease of energy-transfer efficiency as the temperature rises (circles) and the melting of the helix (dashed line). The minimum of the former curve at 30 °C is evident. Note that the zero value at 30 °C is not exact. It is clearly a minimum near 30 °C, but the zero values rests upon the apparent absence of any discernible bump in the spectrum at 265 nm in Figure 2a. The error bars of Figure 4 properly indicate the magnitudes of both the random error (spectral
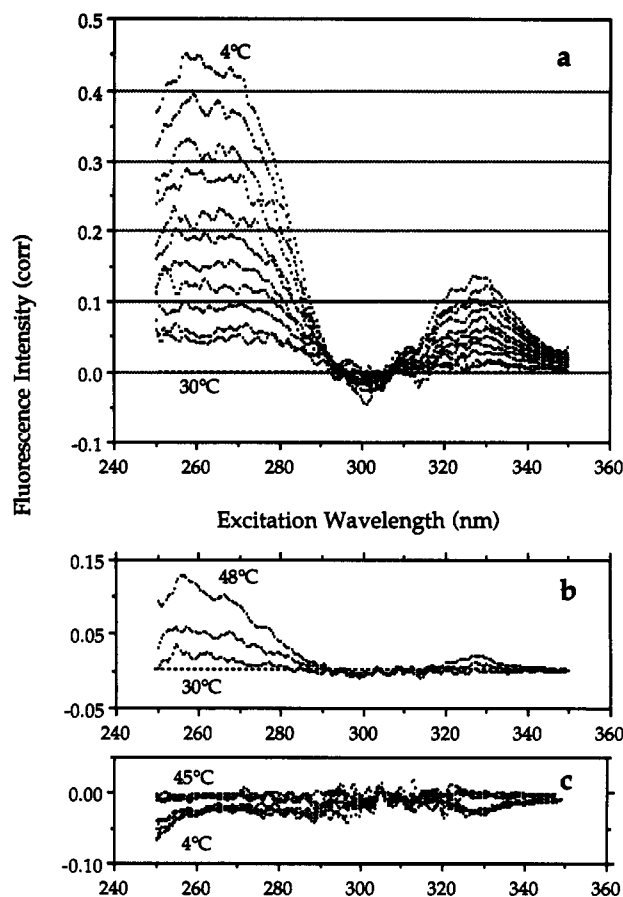

FIGURE 3: Difference fluorescence excitation spectra vs temperature. d[CTGA(2AP)TTCAG]₂ DNA decamer. The spectrum at 30 °C was subtracted from the spectra at each temperature from 4 to 48 °C, giving, approximately, the difference between the spectrum at that temperature and the spectrum corresponding to no transfer. (a) 4–30 °C. (b) 30–48 °C. (c) Equivalent spectra of 2-aminopurine free base; 10 spectra; 4–50 °C.
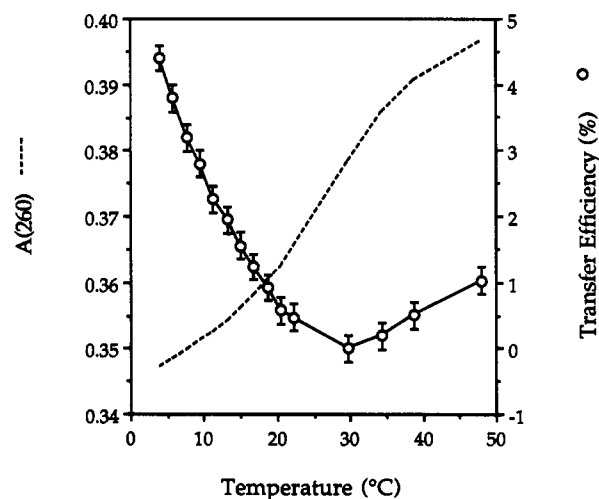

FIGURE 4: 260-nm absorption vs temperature (dashed line) and energy-transfer efficiency calculated from the temperature-dependent fluorescence excitation and absorption spectra of d[CTGA(2AP)-TTCAG]₂ using eq 3 (circles). See text. Solution conditions for fluorescence as in Figure 2.

noise) and the uncertainty of the absence of a 265-nm band at 30 °C.

The increase of the 260-nm absorbance upon helix melting gives a possible trivial explanation for the apparent 260-nm excitation peak minimum at 30 °C (Figure 3). Since the 260-nm absorbance is increasing as the temperature rises, it

could possibly offset the decreasing transfer efficiency. Equation 3, however, and thus Figure 4, has already taken this temperature-dependent absorbance into account. The true transfer efficiency reaches a minimum and increases again above 30 °C. The 30 °C transfer minimum is thus not explained by hypochromism at 260–270 nm.

DISCUSSION

Energy-Transfer Efficiency. The observation of an excitation peak for the 2-aminopurine emission in the 260–270-nm region in the d[CTGA(2AP)TTCAG]$_2$ duplex decamer below the melting temperature is a simple, clear demonstration of the existence of excitation energy transfer from the normal DNA bases. The data show that transfer occurs most efficiently at the lowest temperatures when the DNA is double-stranded. In the double-stranded form, the bases are hydrogen-bonded and stacked most closely, thus facilitating transfer from base to base. The magnitude of the energy-transfer efficiency is not large, decreasing from a maximum of 4–5% at 4 °C as the temperature rises and the helix melts. The data of Figure 4 suggest that the efficiency will increase further if measurements could be extended significantly below 4 °C. If the transfer efficiency is proportional to the fraction of DNA in the double-helix state, this increase cannot be large. The apparent minimum at 30 °C and the small rise at higher temperatures, when the helix is completely melted, show that the duplex structure facilitates transfer but is not a requirement for transfer. The absence of transfer near 30 °C is supported by the absence of any apparent peak in the excitation spectrum near 270 nm at 30 °C.

Base stacking is expected to decrease as temperature increases and the double helix changes to single strands, though single strands can indeed have stacked bases (Sänger, 1984). The data show that at the high-temperature limit energy transfer is not zero, supporting the assertion that the single-stranded bases are stacked or otherwise oriented to allow transfer. One might expect that the increased mobility of the bases in the single-strand form allows the bases to transiently pass through conformations favorable to energy transfer and for photodimer formation (Patrick & Rahn, 1976). If the limiting, low-temperature, "motionless" DNA conformation is best for transfer and if the limiting value of transfer efficiency is 10%, then in the high-temperature, high-mobility, single-stranded form ($\epsilon_t \sim 1\%$ at 50 °C) the bases occupy this "best" conformation about 1%/10% = 0.1 or about 10% of the time. Temperature-dependent mobility is also likely a factor in the decrease of transfer efficiency from 4 to 30 °C. Of all the conformations which actually occur during the conformational transition from the B helix to the melted duplex, if the B-helical (or other low-temperature-limit) structure is best for transfer, then any transient motion away from this structure will reduce transfer, as observed from 4 to 30 °C.

A second possible cause of the increase of transfer with decrease of temperature is an excited-state lifetime increase. Generally, the probability of transfer will be proportional to the lifetime of the donor in the absence of the acceptor. The fluorescence decays of normal bases in the adenine- or 2AP-containing decamers have not been measured. In the temperature range 4–50 °C, these lifetimes are likely multiexponential, with primary decay components of lifetime tens of picoseconds (Georghiou et al., 1985; Rigler et al., 1985; Nordlund, 1990). The temperature dependence of the fluorescence lifetime or the yield of an isolated nucleotide can be approximately described by the activation energy of an internal conversion quenching rate (Gueron et al., 1974). If

energy transfer below about 10 °C is governed by donor lifetime effects, the transfer efficiency should, for low efficiencies, be proportional to the lifetime. A plot of log efficiency vs $1/T$ should then give the activation energy of the quenching rate. Such a plot of our data below 16 °C yields an activation energy of about 17 kcal/mol. Gueron et al., on the other hand, observed an activation energy of 3–4 kcal/mol for the temperature dependence of the fluorescence yield of TMP in ethylene glycol/water mixtures from 150 to 250 K. We thus believe temperature-dependent lifetime effects do not appreciably affect energy transfer in the temperature range 4–50 °C.

Wavelength Dependence and Identity of the Donor. The excitation spectrum of the energy-transfer band should coincide with the absorption spectrum of the donor in the simplest case. In actuality, excitation spectra are often shifted to the red compared to the absorption spectrum, as has been shown for DNA bases, both normal and modified (Aoki & Callis, 1982; Ge & Georghiou, 1991b; Evans et al., 1992), and transfer efficiency may be more efficient for shorter-wavelength excitation, particularly if transfer occurs before the excited state is vibrationally relaxed. In the case of normal DNA bases, absorption peaks of individual monodeoxynucleotides at neutral pH are approximately the following: A, 260 nm; T, 266 nm; G, 252 and 275 nm; C, 270 nm. The maximum in the excitation spectrum (Figure 3a) lies between 260 and 270 nm, but the intensity is within 10% of the maximum from 255 to 270 nm, so an unambiguous identification of the particular base or bases acting as donor cannot be done.

Transfer Distance and Rate. If 5% of the energy absorbed by all normal bases is transferred to 2AP, if the average excited-state lifetime of the normal bases in DNA is 10 ps (Georghiou et al., 1985), and if all DNA molecules are duplex, then the overall rate at which energy is transferred to 2AP is $k_t \approx (0.05)(10^{11}\,\text{s}^{-1}) \approx 5 \times 10^9\,\text{s}^{-1}$. This rate is quite small compared to estimates from standard transfer theories (Gueron, 1974; Georghiou et al., 1990), unless many transfer steps take place before the deposition of the energy on 2AP. If, however, only the energy from a single, directly-excited DNA base can be transferred, e.g., from the thymine base hydrogen-bonded to 2AP, then the efficiency of this single T → 2AP transfer is $5\% \times (9/1) \approx 45\%$, and the rate would be about $5 \times 10^{10}\,\text{s}^{-1}$, since this T is one of nine normal bases per strand. Primarily nearest-neighbor singlet transfer was inferred by Georghiou et al. in methylated DNA (Georghiou et al., 1990). A clear resolution of the transfer distance and rate question awaits experiments on DNA of varying length and more accurate calculations of the electronic excited states and interactions in DNA. One can state with certainty, however, that transfer occurs from bases which are at most five steps removed from 2AP and that the overall transfer efficiency is low but measurable.

Temperature Dependence of Transfer. The transfer efficiency decreases rapidly as the temperature rises from 4 to 30 °C, at a lower temperature than does the helix melting. The spectroscopic shift of the 2-aminopurine base with temperature has also been observed to exhibit this behavior. At 4–10 °C the large majority of DNA molecules are in the duplex form, yet the increase in transfer efficiency from 10 to 4 °C is significant. This implies that transfer efficiency is not simply determined by duplex vs single-strand fraction. DNA bases exhibit local mobility which increases with temperature even in the double-stranded form. Such mobility has been demonstrated for the 2AP base in this *EcoRI* recognition sequence oligonucleotide (Gräslund et al., 1987;

Nordlund et al., 1989). Since transfer will be optimal at some particular orientation of the 2AP base with respect to the other bases, any motion of the bases away from that orientation can only result in a decrease of transfer. It appears, then, that transfer is more efficient in the B-helical, duplex conformation than in any of the other actually-occurring conformations, particularly when conformational mobility is low. From the appearance of the low-temperature data in Figure 4, further reduction of the temperature (below 4 °C, if that were possible) would result in no appreciable increase in the proportion of duplex but could significantly increase the transfer efficiency, presumably via further reduction of mobility.

Is Transfer Low because of the Modified 2AP Base? Besides acceptor–donor distance, two primary factors govern energy transfer in the standard treatment of energy transfer: (i) overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor and (ii) the relative orientation of the donor and acceptor dipoles (Förster, 1948). The room temperature emission spectra of normal DNA bases peak in the 330-nm and longer wavelength region (Aoki & Callis, 1982; Ballini et al., 1982; Rigler et al., 1985; Georghiou et al., 1990). The overlap of this emission with the absorption of 2AP (306–315 nm) (Evans et al., 1992) is much better than with other normal bases (260–290 nm) and would tend to increase the transfer efficiency to 2AP compared to that to normal bases. The orientations of the excitation and emission dipoles of 2AP are presently unknown, so no definitive conclusions can be drawn about orientation effects on transfer. Nevertheless, the transfer efficiencies measured in the present work under the assumption of single-step transfer are comparable to those measured for methylated DNA (Georghiou et al., 1990), after accounting for the greater number of acceptors in the latter case. Transfer is thus not low because of the chemical nature of the 2AP base.

Implications for UV Damage to DNA. Energy transfer from normal bases to extrinsic fluorescence probes, highly modified bases, and methylated bases has been observed in RNA and DNA of various forms. However, in only two cases, methylated DNA (Georghiou et al., 1990) and poly(dG-dC)·poly(dG-dC) (Huang & Georghiou, 1992), is the transfer evidence direct and directly applicable to transfer of excitation to a potential site of UV damage in DNA of "normal" structure. The present results confirm that UV singlet excitation energy transfer is a possible mechanism for movement of energy in DNA. Transfer is most efficient in the B double-helical form and becomes more and more efficient as the temperature goes down. This contrasts with the constant thymine dimer formation probability vs temperature below the melting temperature (Patrick & Rahn, 1976). In view of the rather low observed transfer efficiency, it is doubtful that transfer over more than a few bases occurs or has a significant role in the localization of any of the major types of UV damage sites in native DNA.

ACKNOWLEDGMENT

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