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regarding pathway evolution had been made along two lines. Simple (exponential) distance dependencies observed in the photosynthetic proteins led Moser, Dutton, and co-workers to suggest that evolution manipulates ET rates using \( R_{DA} \) and Marcus (nuclear) parameters (4). Gray, Winkler, and co-workers, in contrast, argued that strong pathways have evolved to accelerate ET in some proteins (5). Indeed, the structure of common biological redox cofactors seems to permit ET proteins to access both mediation regimes.

We suggest that, in the multiple-pathway regime, the evolutionary linkage between the specific protein fold and the ET rate is likely to be weak: In this regime, \( R_{DA} \) determines tunneling propensity. In the single-pathway large \( C \) regime, however, ET kinetics and protein structure are strongly linked. Although the Ru proteins only display slow rates in the dominant pathway regime, either strong or weak coupling pathways could arise in the dominant pathway regime, generating order of magnitude effects on the ET kinetics from protein structure. This structure-function perspective extends the pathway-evolution conjecture of Ramirez et al. (5), by accounting for the influence of thermal motion on the protein-mediated coupling, and also suggests that the Moser-Dutton (average-medium) view is valid in the multiple-pathway regime common to many large edge-coupled redox cofactors. Tunneling routes involving axial ligands seem to be the most likely candidates for kinetics that is sensitive to coupling pathway structure [e.g., the heine a to heime \( a_1 \) pathways in cyt c oxidase (5–6)]. How often and where nature has used pathway-specific or multiple-pathway regimes remain to be determined by future analysis and experiments. Also, in the small \( C \) regime, proteins will have ET kinetics that are robust to modifications of single-pathway links (e.g., by manipulating hydrogen bonding), whereas pathway structural changes in the large \( C \) regime may have a larger influence on ET kinetics (30–32).

References and Notes

24. This is one of a number of methods being developed with few adjustable parameters that may be used to compute coupling interactions in systems as complex as proteins. For related studies, see V. Barone, M. D. Newton, R. Imprata, Chem. Phys. Chem. 7, 1231 (2006) and T. Kawatsu, T. Kakitani, T. Yamato, J. Phys. Chem. B 106, 11356 (2002).
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Thymine Dimerization in DNA
Is an Ultrafast Photoreaction

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Femtosecond time-resolved infrared spectroscopy was used to study the formation of cyclobutane dimers in the all-thymine oligodeoxynucleotide (dT)\(_{18}\) by ultraviolet light at 272 nanometers. The appearance of marker bands in the time-resolved spectra indicates that the dimers are fully formed ~1 picosecond after ultraviolet excitation. The ultrafast appearance of this mutagenic photolesion points to an excited-state reaction that is approximately barrierless for bases that are properly oriented at the instant of light absorption. The low quantum yield of this photoreaction is proposed to result from infrequent conformational states in the unexcited polymer, revealing a strong link between conformation before light absorption and photodamage.

The most abundant lesion in ultraviolet (UV)–irradiated DNA is the cyclobutane pyrimidine dimer (CPD) that is formed between adjacent thymine bases (Fig. 1) (1). This mutagenic photoproduct disrupts the normal cellular processing of DNA and leads to a complex web of biological responses, including apoptosis, immune suppression, and carcinogenesis (2–4). Organisms possess elaborate repair pathways to counter this constant threat to genomic integrity. Aside from their biological importance, CPDs are of interest as structural reporters. Thymine-dimer yields are not the same at all TT doublets in a given DNA sequence, but these yields depend, in poorly understood ways, on the identity of the flanking bases and on local conformation (1). By exposing DNA to UV light and then measuring the relative photoproduct yields with single-nucleotide resolution, it has been possible in favorable cases to obtain structural information (5–7). In order for this methodology to achieve its full potential, molecular-level understanding of the dimerization mechanism is essential. We report a dynamic study of thymine dimerization that provides insight into the coupling between DNA structure and DNA photodamage.

CPD formation is a \([2+2]\) photocycloaddition reaction in which the carbon-carbon double bonds of proximal pyrimidine bases react to form a cyclobutane ring. In the analogous reaction between two ethylene molecules, electronic excitation and the proper orientation of the reacting double bonds are needed for the reaction to occur (8). Unlike the case of free ethylene molecules, pyrimidine bases in DNA are tethered to the sugar-phosphate backbone, and this tethering restricts the achievable orienta-
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Some conformations are simply impossible because of backbone constraints. Thus, a single CPD isomer (the cis-syn isomer shown in Fig. 1) is formed in UV-irradiated oligo- and polydeoxyribonucleotides, whereas two thymine molecules diffusing freely in aqueous solution yield all six stereoisomers (1). Because DNA is moderately flexible, a vast number of conformations exist. Some of these have the bases positioned favorably for a reaction, whereas others do not. DNA is highly dynamic, and motions such as the stacking and unstacking of bases, base-pair breathing and opening, torsional oscillations, and helix bending will incessantly bring a given bipyrimidine doublet into and out of favorable geometries for dimerization. The impact of these motions on the reaction kinetics depends on how their rates compare to the rate of reaction by favorably oriented bases (9). Direct kinetic measurements of dimerization can thus elucidate the potentially complex interactions between conformational dynamics and photodamage.

In an excited-state reaction, motion along the reaction coordinate occurs in competition with energy-wasting steps such as fluorescence and internal conversion to the electronic ground state. In the past few years, it has become possible to directly observe the dynamics of excited electronic states in DNA model compounds by femtosecond spectroscopy (10, 11). It has been proposed that the very high rate of nonradiative decay by the singlet $\pi^* (\pi^* \pi^*)$ states of single nucleobases can greatly restrict photodamage (10). However, recent work has revealed the presence of additional, rather long-lived singlet states in DNA (11) and single bases (12). In oligodeoxyribonucleotides, lifetimes of $<$1 ps to $>$100 ps have been observed, depending on base stacking and base sequence (11). Additionally, at least 10% of all singlet excitations in single pyrimidine bases such as thymidine 5'-monophosphate (TMP) decay to singlet $\pi^* (\pi^* \pi^*)$ excited states with lifetimes in excess of 10 ps (11). Kinetic measurements can determine which of these diverse excited states is the dimer precursor.

Past efforts to observe dimerization kinetics have been unsuccessful. It has been shown by flash photolysis that photodimers are formed in the all-thymine oligodeoxyribonucleotide (dT)$_{20}$ in <200 ns, the time resolution of the laser system that was used (13). Femtosecond transient electronic spectroscopy (11) has not provided direct evidence for dimer formation because CPDs do not absorb at wavelengths longer than ~270 nm. Because of its chemical bond specificity, vibrational spectroscopy can often unambiguously identify transient species and stable photoproducts (14). We therefore recorded time-resolved infrared spectra of a DNA model compound that was excited by a femtosecond UV pump pulse (15). The system studied was single-stranded (dT)$_{18}$, which was chosen in order to maximize the number of dimers that were formed with each laser pulse. In this DNA model system, every absorbed photon excites a residue that is capable of dimerization. Quantum yields in the closely related systems poly(dT) (0.033) (16) and (dT)$_{20}$ (0.028) (13) are among the highest reported for any DNA compound. In contrast, the dimerization quantum yield is over 30 times lower in double-stranded genomic DNA (17). This large reduction is due to the low frequency of TT doublets and the absorption by nonthymine bases in mixed-sequence DNA. After presenting our results for (dT)$_{18}$, we will discuss the implications for double-stranded nucleic acids.

Steady-state infrared (IR) absorption spectra of (dT)$_{18}$ in D$_2$O were recorded before and after UV irradiation at 266 nm, in order to locate IR marker bands that were indicative of dimerization. In the spectrum obtained before UV irradiation (black curve in Fig. 2A), three strong bands were observed at 1632, 1664, and 1693 cm$^{-1}$. These bands, which arise from double-bond stretches associated with the two carbonyl groups and the C5=C6 double bond (18), bleached strongly after several minutes of UV exposure (Fig. 2A). Difference spectra were calculated by subtracting the steady-state IR spectrum from each spectrum of the UV-irradiated oligomer (Fig. 2B). Negative bleaching signals were apparent in the double-bond stretching region above 1600 cm$^{-1}$. In addition, positive peaks between 1300 and 1500 cm$^{-1}$ grew in with increasing exposure time. The IR absorption spectrum of the photoproduction (solid curve, Fig. 2C) was obtained from the difference spectra in Fig. 2B by target analysis (19), assuming that a single photoproduct is formed. In fact, a pyrimidine (6-4) pyrimidone photoproduct is also generated at TT doublets, but it is not in polymeric DNA. Fractions of new bonds shown in red) if a reactive conformation is present at the time of excitation.
can be neglected because its quantum yield is 50 times lower in poly(dT) (20). The absorption spectrum (Fig. 2D) of a previously described model compound of the cis-syn thymine dimer (21) is in excellent agreement with the solid trace in Fig. 2C, showing that this is the only dominant photoprocess under these conditions. Bands in the dimer spectrum substantially overlap those of unirradiated (dT)18 above 1500 cm⁻¹. In contrast, a trio of marker bands is evident at 1320, 1402, and 1465 cm⁻¹ (Fig. 2C), and these bands became the focus of the time-resolved experiments.

Broadband IR transient absorption signals were recorded between 1300 and 1550 cm⁻¹ after excitation of (dT)18 by a femtosecond pump pulse at 272 nm (17). For comparison, measurements were carried out on TMP, which cannot dimerize on the time scales of interest here because of the slow rate of diffusion-controlled encounter by two TMP molecules. Transient spectra measured for both solutes are compared side-by-side in Fig. 3. Negative absorbance changes (bleaches) are colored blue, whereas positive signals are red. The bleaches monitor the repopulation of the starting material, whereas positive signals arise from the vibrational bands of excited states or photoproducts. At first glance, the transient IR spectra of TMP and (dT)18 are very similar. The quantum yield for dimerization in (dT)18 is just 2 to 3%, and most excitations in both systems decay nonradiatively on similar time scales (II).

Dynamic events revealed by the time-resolved spectra in Fig. 3 that are common to both solutes are discussed first. Initially, UV excitation populates the lowest-energy ππ* state, resulting in bleaches at frequencies corresponding to ground-state vibrations (dashed gray lines in Fig. 3). These bleaches have their maximum amplitudes near time zero, as seen in the spectra recorded 0.48 ps after the pump pulse. Positive signals are seen at this time at all frequencies where bleaching is not observed. These broad bands decay with a lifetime close to that of the ππ* state (540 fs for thymidine (10)) and are no longer present in the 3.3-ps spectra. The short lifetime of this state is limited by internal conversion, which moves the population nonradiatively from the ππ* state to the vibrationally excited electronic ground state. The photon energy is thus converted into sudden vibrational heating. This produces positive bands on the red edge of the negative bleach signals, resulting in distinctive sigmoidal line shapes (22) such as the one seen near 1480 cm⁻¹ in the 3.3-ps spectra. These features disappear by vibrational energy transfer to the solvent (vibrational cooling), with a time constant of 2 to 4 ps (11, 14), and are no longer visible at 20 ps.

The bleach near the maximum of each vibrational mode recovers in multiexponential fashion with similar kinetics as those that were previously recorded by transient absorption signals at UV wavelengths (11). The decay is 85 to 90% complete within 10 ps, whereas the remainder of the bleach recovers with time constants that vary between 100 and 1000 ps because of the decay of the ππ* population (12). A broad positive band near 1350 cm⁻¹ decays on a 100-ps time scale and is tentatively assigned to this ππ* state. The spectra at 3 ns (Fig. 3) is dominated by a broad sigmoidal line shape, extending from 1300 to 1800 cm⁻¹. This distinctive signature arises from a temperature-jump effect, which is described in the supporting online material (SOM) in more detail. The hot-water contribution to the transient spectrum appears within a few picoseconds, but it then remains constant in our time window because of slow heat transport out of the laser focus (23).

There are subtle but significant differences between the time-resolved IR spectra in Fig. 3. Greater modulation in the 20-ps and 3.5-ns spectra for (dT)18 is due to absorption in the oligomer at each of the three marker-band frequencies that are identified in Fig. 2C. The difference is readily seen in a comparison of the transient spectra that are recorded for the two samples at a 3-ns delay time in the top panel of Fig. 4A.

The red trace in Fig. 4A is the difference spectrum calculated by subtracting the ground-state absorption spectrum of (dT)18 from the dimer spectrum of Fig. 2C. This trace represents the expected absorption changes induced by dimer formation. The transient difference spectra at 15 ps and 3 ns show positive peaks at each of the dimer marker-band frequencies and contributions from ground-state bleaching. The excellent agreement with the stationary spectrum shows unequivocally that thymine dimers are present ~15 ps after excitation.

The dynamics of the marker bands at earlier times can be seen in a contour plot of the transient difference spectrum between 1 and 25 ps (Fig. 4B). The positive marker bands at 1402 and 1320 cm⁻¹ are clearly visible over the entire time range. The marker band at 1465 cm⁻¹ is visible down to 4 ps, but it is obscured by vibrational cooling of hot thymine molecules at earlier delay times. Because TMP and (dT)18 exhibit different cooling dynamics (11), the vibrational cooling signatures do not fully cancel each other and instead show up in the difference plot in the vicinity of intense ground-state bands. Thus, the cooling dynamics from the hot 1480 cm⁻¹ band (Fig. 3) cover the 1465 cm⁻¹ marker band at early delay times. Cooling is also seen at other wavenumbers during the first few picoseconds; e.g., around 1350 cm⁻¹. For delay times <1 ps, the signals are dominated by ultrafast relaxation of the electronically excited state, which obscures direct observation of dimer formation at the shortest times. Nevertheless, the observation of the dimer marker bands 1 ps after light absorption indicates that the reaction occurs on a femtosecond time scale.

The dimer yield can be estimated from the average amplitude of the marker bands in Fig. 4 of ~30 μ-optical density (OD) units. This is 3% of the initial bleach of 1 mOD that was seen 1 ps after photocexcitation at 1480 cm⁻¹. This band has a cross section comparable to that of the three marker bands, so the reported dimerization yield of 2 to 3% (13, 24) should produce a signal of 20 to 30 μOD, as observed. The dimer yield at ~1 ps thus equals the value from steady-state experiments within experimental uncertainty, demonstrating that dimerization is
an ultrafast photoreaction. The high speed of this bond-forming reaction is noteworthy but not unprecedented. Ultrafast reaction rates are seen for some bimolecular reactions when the reactants are suitably preoriented (25). Also, the closely related intramolecular [2+2] photocycloaddition reaction of norbornadiene occurs in the gas phase in <100 fs (26).

The ultrafast time scale of thymine dimerization suggests that an essentially barrierless path connects the initial \( ^1\pi\pi^* \) state with the end product. This suggests that a conical intersection lies along this path as in computational studies of other pericyclic photoreactions (8). Dimerization in (dT)\(_{18}\) occurs more rapidly than many motions that could bring poorly oriented bases into a more favorable conformation for reaction. For example, base stacking and unstacking in thymine oligomers require tens of picoseconds, according to a molecular-dynamics study (27). Dimerization thus occurs only for thymine residues that are already in a reactive conformation at the instant of excitation (28, 29). Excited states of unfavorably oriented thymines are quenched before a change of conformation can occur. The extent of dimerization under steady-state irradiation thus depends on the fraction of time that a given doublet spends in reactive versus nonreactive conformations. Control of CPD formation by ground-state structure is fully consistent with the rapid saturation of CPD formation in poly(U) and poly(dT) in a rigid glass at 77 K as compared to room-temperature aqueous solution (30). This occurs because there is a finite number of reactive conformations in the low-temperature polymer, but the polymer in room-temperature solution is able to thermally fluctuate, allowing new reactive conformations to appear as exposure continues.

Because the rate of reaction by favorably aligned thymines is much faster than the rate of conformational change, the quantum yield is equal to the fraction of reactive conformations multiplied by the probability that a reactive conformation dimerizes upon excitation (9). The latter quantity is unknown, but it is likely to approach unity based on the high quantum yields of dimerization in molecular crystals of some pyrimidine bases (31) and in dimers split in rigid matrices (32). With this assumption, the quantum yield for dimerization is simply the fraction of favorably oriented conformations. The low yields for all-thymine oligomers thus reveal that only a few percent of the TT doublets are favorably positioned for reaction at the time of excitation. This finding is consistent with the disordered structure of this rather flexible oligomer (33).

Excited-state modeling is needed to fully characterize the reactive conformations, but some geometrical requirements are readily anticipated. Base stacking, which has been discussed in the past as a necessary criterion for reaction (30), reduces the distance between C5=C6 bonds as compared to an unstacked geometry. However, the dimer geometry suggests that a low value of the dihedral angle between the reacting double bonds may also be important. The conformational changes, such as partial helix unwinding and bending (34), that are observed near the site of a CPD are likely to be the same ones needed to make a conformation favorable for reaction (7).

We fully expect thymine dimerization to be ultrafast in double-stranded DNA, based on the speed of the reaction in single-stranded (dT)\(_{18}\). Base pairing could affect the rates of nonreactive decay steps such as internal conversion by the precursor excited state, but we consider this to be unlikely, because recent time-resolved measurements show no effects due to base pairing on the dynamics of the excited states in AT-containing oligodeoxynucleotides (11). We conclude that dimerization occurs with equal speed for bipyrimidine doublets in single- and double-stranded contexts, provided that the TT geometry is similar for both contexts. Base pairing, on the other hand, will greatly influence the quantum yields by altering the distribution of conformations. The structures of flexible all-thymine oligomers (27, 33) and double-stranded mixed-sequence DNA differ substantially, yet the quantum yields calculated per photon absorbed by a dimerizable thymine (see SOM) are the same to within a factor of \( \sim 2 \) in room-temperature aqueous solution (17, 35). This means that a small percentage of TT doublets react in double-stranded DNA, even though virtually all doublets are well stacked. We propose that the winding of base pairs around the helix axis [the average twist angle is 36° in the B-type DNA (B-DNA) conformation (36)] keeps the C5=C6 double bonds too far apart. In contrast, although base stacking in single-stranded thymine oligomers is rare, the more flexible backbone does not prevent these rare stacks from adopting conformations that are suitable for dimerization.

A comparison of the literature that describes dimer yields in nucleic acids with A-type and B-type double-helical structures supports the hypothesis that dimerization in double-stranded DNA occurs as a result of uncommon conformations. The rate of dimer formation is decreased by up to a factor of 2 when double-stranded DNA is switched from the B-type to the A-type conformation (37). Even larger protective effects have been observed at TT steps in hairpins with A-type structure (38). The same base pairing is found in both structural classes, and the only difference is the distribution of accessible conformations. This evidence establishes that the conformation controls the reactivity in duplex DNA, just as in single-stranded (dT)\(_{18}\). The average twist angle between successive base pairs differs in A-DNA by only a few degrees when compared to B-DNA, suggesting that the ideal geometries in both helices are nonreactive. Instead, dimerization is proposed to take place at TT steps that deviate in just the right way from the average duplex structure. Thus, the smaller amount of conformational variation in A-type versus B-type structures (36) explains the greater resistance of A-DNA to CPD formation.

The model we have derived from our results implies that static TT conformation (7, 29), and
Single Photon–Induced Symmetry Breaking of H2 Dissociation


H2, the smallest and most abundant molecule in the universe, has a perfectly symmetric ground state. What does it take to break this symmetry? We found that the inversion symmetry can be broken by absorption of a linearly polarized photon, which itself has inversion symmetry. In particular, the emission of a photoelectron with subsequent dissociation of the remaining H2 fragment shows symmetry with respect to the ionic H+ and neutral H atomic fragments. This lack of symmetry results from the entanglement between symmetric and antisymmetric H2+ states that is caused by autoionization. The mechanisms behind this symmetry breaking are general for all molecules.

Symmetries are essential building blocks of our physical, chemical, and biological models. For macroscopic objects, symmetries are always only approximate. By reducing the complexity in the microcosm, these symmetries often become strict. Thus, in any symmetric molecule, the ground state has a well-defined parity. This property has far-reaching consequences, such as truncation of rotational spectra or the existence of ortho- and para-molecular isomers (J). One way to break the symmetry is isotopic substitution of one of the nuclei (2). In larger systems, symmetry breaking can also be achieved through selected vibrational modes, such as asymmetric stretch, which lies at the origin of the Jahn-Teller and Rener-Teller effects (3). Alternatively, external fields can be used to favor a particular molecular direction, a method that has recently been used by Kling et al. (4) to induce asymmetric dissociation of the H2+ molecular ion into a proton and a hydrogen atom. Here, we show that, in dissociative ionization by absorption of a single photon

\[ hv + H_2 \rightarrow p + H + e^- \]  

symmetry breaking is possible even in the absence of an external field. This is the smallest and most fundamental molecular system for which such symmetry breaking is possible. Symmetry operations in a molecule that has a well-defined parity can change the sign of the ground state wave function (odd parity, or ungerade, states). However, all observables must be symmetric, because they are squares of wave functions or transition matrix elements. To achieve left-right asymmetry in an observable, the system must be put into a coherent superposition of gerade (g) (even) and ungerade (u) (odd) molecular states. The relative phase between the two states can then lead to a left or right localization of an electron. Direct photoionization usually cannot induce this outcome, because the g and u states of the remaining molecular ion have different energies. Therefore, two ionization pathways are distinguishable by the electron energy and hence the coherence is lost.

Figure 1A shows the energy diagram for the H2 and H2+ molecules. The energy difference between the lowest g and u states in H2+ − 2\( \Delta E_u^{g} \) (1s\( g \)) and − 2\( \Delta E_u^{u} \) (2p\( u \)), respectively, is about 17 eV in the Franck-Condon region of H2. Thus, if H2 is directly ionized in a vertical transition by a photon of energy \( h \nu \), the photoelectron will have an energy of about \( E_{e} = h \nu - 16 \) eV when the remaining H2+ is in the g state, whereas it will have \( E_{e} = h \nu - 33 \) eV when the remaining H2+ is in the repulsive u state. Both ionization paths are distinguishable by the energy (Fig. 1, B and C). Because in either path H2+ is in a state of well-defined parity, it manifests no memory of the direction toward