What Is Adenine Doing in Photolyase?

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The short answer to the title question is that it acts as an electrostatic bouncer that shoves the charge flow from flavin toward the DNA lesion that photolyase repairs. This explanation is provided by an explicit time-dependent quantum mechanical approach, which is used to investigate the electron transfer process that triggers the repair mechanism. The transfer occurs from the flavin photolyase cofactor to the cyclobutane ring of DNA, previously formed by light-induced cycloaddition of adjacent pyrimidine bases. The electron wave function dynamics accurately accounts for the previously proposed mechanism of transfer via the terminal methyl group of the flavin moiety present in the catalytic electron-donor cofactor, $FADH^-$, which also contains adenine. This latter moiety, which has often been assumed to be present mainly for structural reasons, instantaneously modifies the interaction between acceptor and donor by a variation of the electrostatic interactions so that the presence of its local atomic charges is necessary to trigger the transfer. In principle, knowledge of the details of the electron transfer dynamics and of the important role of polarization effects can be exploited to improve the efficiency of the repair mechanism in artificial systems.

Introduction

Photolyases are proteins that bind to UV-damaged DNA and repair lesions where two adjacent pyrimidine molecules, usually thymines, form a cyclobutane ring.[1–8] In the cascade of events that lead to the opening of the ring, a doubly reduced flavin adenine dinucleotide, $FADH^-$, is first indirectly photoexcited and then transfers an electron from a $\pi \rightarrow \pi^*$ singlet state to the lesion. The $FADH^-$ cofactor is deeply buried in a α-helical domain of the protein and has a U-shaped conformation with isoalloxazine and adenine rings in close proximity.

Two pathways for the electron transfer (ET) have been suggested: the direct one, where the electron is transferred from the terminal methyl group of flavin to the carbonyl group of a thymine,[8,9] and the indirect one, where the electron is ultimately transferred from the amino group of adenine to the carbonyl groups of the thymine dimer.[10,11] The indirect ET channel is supported by crystallographic data:[8] the U-shaped cofactor has an amino group located in a favorable position for ET to the pyrimidines. However, the lower adenine redox potential (compared to the thymine redox potential) hinders the efficiency of the transfer. Femtosecond time resolved florescence experiments excluded a direct role for adenine and demonstrated that the transfer process occurs via the terminal methyl group of the flavin cofactor.[9]

Prytkova et al.[12] calculated the energies and nature of the lowest excited singlet states of $FADH^-$ with different levels of theory and showed that its two lowest $\pi \rightarrow \pi^*$ singlet states are localized on the part of the flavin ring that is closer to the lesion. The localization of the excited state of the donor enhances the donor−acceptor coupling and the direct through-space ET between $FADH^-$ and the dimer. They calculated the coupling between the lowest-energy donor excited state of $FADH^-$ and the acceptor states of the thymine dimer, and averaged the results over molecular dynamics (MD) runs. The subsequent analysis of the coupling found that adenine is not essential to the tunneling, and the electron of the excited state tunnels to the dimer through the C8-methyl group that is in close vicinity of the dimer.

Prytkova et al. did not completely rule out superexchange contribution from adenine, but ascribed the role of adenine to the stabilization of the dimer−$FADH^-$ system inside the active site and to the anchoring of the dimer to $FADH^-$ by formation of hydrogen bonds between adenine and the dimer.[8] Electron paramagnetic resonance/density functional theory (EPR/DFT) calculations conducted by Weber et al.[13] on the electronic structure of the neutral flavin radical $FADH^{\cdot}$ provided evidence for a superexchange-mediated mechanism in the ET process via the adenine moiety, in which intermediate electronic states of the adenine cause an effective coupling between the involved donor and the acceptor species.

In this work, we are interested in the ET mechanism between $FADH^-$ and the damaged dimer of two thymines. We examine the role of all the components that govern the transfer with a model that explicitly calculates the dynamics of the electron (or better, its wave function) in time. The information available to date, that is, the value of the matrix element for the transfer on the order of 0.5 meV, does not show how the flow of charge goes through the methyl group, and more importantly, it does not find a role for adenine, which is suggested to be present only for structural reasons. Apart from the general interest of the issues, knowledge of the details of the electron dynamics can be exploited to improve the efficiency of the repair mechanism in artificial systems.

Structural and Computational Background

The geometry used in this work was obtained from the crystallographic structure[8] of a complex formed by the *Anacystis nidulans* DNA photolyase and a 14-nucleotide oligomer DNA duplex containing a thymine dimer.[8] When photolyase complexes DNA, the pyrimidines are specifically recognized and

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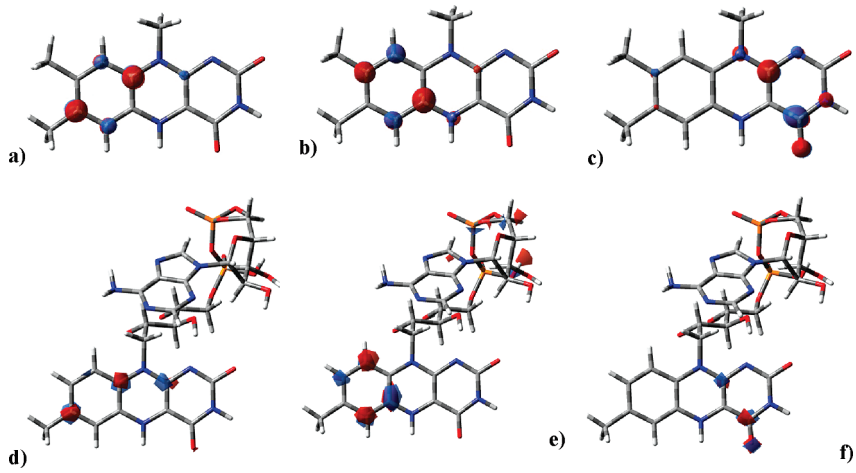

Figure 1. Initial orbitals at time $t = 0$. The wave functions were obtained at the B3LYP/6-31G* level in the absence of the thymine dimer (cyclobutane lesion) and resemble those of ref 12. (a,b,c) LUMO, LUMO+1, and LUMO+2 calculated after removing ribytol and adenine from the crystal structure of $FADH^-$ and replacing them by a methyl group. (d,e,f)The same orbitals of the entire $FADH^-$ calculated by substituting the terminal CH_3 group (through which the transfer occurs) by a hydrogen atom.

flip out of the DNA duplex in order to enter the active side of the protein. Unwinding of the DNA helix creates a hole in the double helix of roughly 10×10 Å^2. Unfortunately, the crystallographic structure with the thymine dimer in the favorable position for the ET process cannot be used as it is. The strong synchrotron radiation used in the experiments probably promoted the cyclo-opening reaction, even though the two bases retain a structure close to what they have in the cyclobutane ring.

The AMBER8 package[14,15] was used to modify the structure and form the cyclobutane ring. A subsequent B3LYP/6-31G*[16] optimization was used to calculate accurate bond lengths of the cyclobutane fragment.

A quantum-mechanical time-dependent propagator was used to evolve the electronic wave function of the donor $FADH^-$ system and produce the time-dependent ET pathway. Integration of the Schrödinger equation by using finite difference approaches tends to produce numerical problems. Here, we exploit the stable algorithm proposed by Allen and co-workers,[17–20] which reads

$$\psi(t + \Delta t) = \left(1 + \frac{iH\Delta t}{2\hbar}\right)^{-1}\left(1 - \frac{iH\Delta t}{2\hbar}\right)\psi(t) \qquad (1)$$

where ψ is the electronic wave function, which is not in a stationary state and is effectively a wavepacket. At $t = 0$, ψ is localized on flavin; H is the electronic Hamiltonian matrix of the entire system (flavin, adenine, and pyrimidine dimer), and Δt is the time-step, set to 2.0 atomic units, or 0.048 fs. The nuclear geometries are frozen during the electron wavepacket dynamics. The initial wave functions $\psi(t = 0)$ and the Hamiltonian, H, were obtained with the Gaussian03 suite of programs at the B3LYP/6-31G* level. Importantly, $\psi(t = 0)$ is not an eigenfunction of H. The coefficients of $\psi(t)$ are updated at each time step, and its overlap with the orbitals of the fragments is calculated. The approach has been used by us to investigate the dynamics of photoinduced dissociation,[21] nonlinear optical properties,[22] and mono and multiphoton excitations.[23]

Results and Discussion

In this work, we focus on the electronic details of the transfer mechanism of the charge from the excited state of $FADH^-$ to the cyclobutane dimer. The intent is to obtain insight (at atomic level) into the time-dependent pathway. For this purpose, the wave function of the excited electron must be described in terms of atomic orbital and be perturbed by the molecular environment. The role of the vibrations, which has been thoroughly studied elsewhere,[12] is not investigated here.

For the sake of generality, we selected six initial orbitals, $\psi(t = 0)$, for propagation of the electron. They were obtained from B3LYP/6-31G* calculations. These wave functions represent the initial, prepared state from where the dynamics starts. All the orbitals were calculated in the absence of the cyclobutane moiety. The first three wave functions, a, b, and c, were determined after removing ribytol and adenine from the crystal structure of $FADH^-$ and replacing them with methyl groups. As shown in Figure 1, they belong to the flavin fragment and are the lowest unoccupied molecular orbitals LUMO, LUMO+1, and LUMO+2. In time-dependent DFT calculations, we found that LUMO and LUMO+1 are the virtual orbitals involved in the excitation to the first singlet excited state of flavin, which is responsible for the photophysics of photolyase. Wave functions d, e, and f were determined for the entire $FADH^-$ upon hydrogen substitution of the methyl group through which the electron propagates to reach the cyclobutane fragment.[12] These orbitals are equivalent to a, b, and c but for a larger (demethylated) system. The selection of these wave functions allows one to independently analyze the two possible ET pathways, direct and indirect, by replacing either a methyl group or the adenine moiety.

In this computational approach, the quantum chemical Hamiltonian matrix represents the system where the propagation takes place. The first, or entire, system is the Hamiltonian of the flavin moiety, the adenine and the cyclobutane fragment. Other reduced systems and augmented systems were also considered. The second system consists of $FADH^-$ (the flavin moiety, the rybitol, and the adenine) and the cyclobutane unit, but a hydrogen atom replaces the methyl group of flavin through which the transfer occurs. The third system consists of flavin, and cyclobutane; the adenine and the rybitol have been removed. The fourth system has adenine represented by the partial charges of its atoms. The fifth system has adenine represented only by the local charges of its NH_2 fragment. The sixth system augments the first one by addition of the partial charges of the photolyase protein.

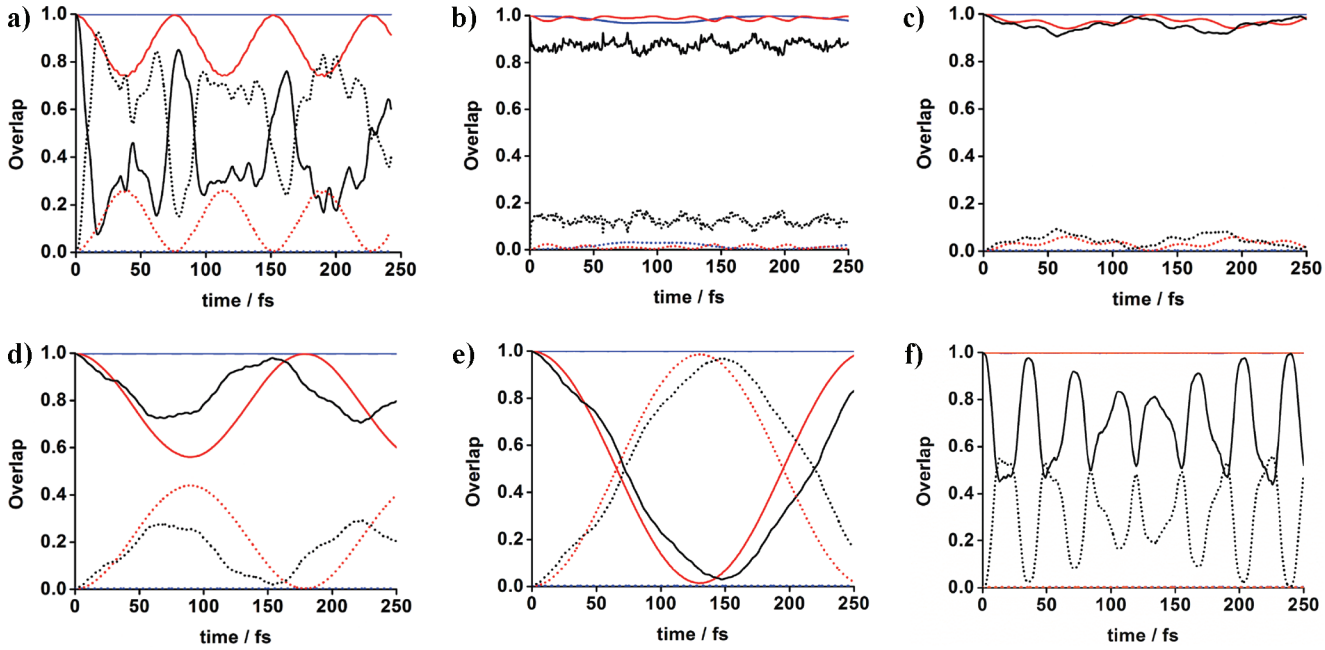

Figure 2. Blue for LUMO, red for LUMO+1, and black for LUMO+2. Overlaps of the evolving wave functions are referred to the donor (flavin moiety, initial overlap equal to 1, solid line) and acceptor (cyclobutane moiety, initial overlap equal to 0, dotted line): (a) dynamics of wave functions a, b, and c of Figure 1 in the entire system; (b) dynamics of wave functions d, e, and f of Figure 1 in the entire system plus rybitol; (c) dynamics of wave functions a, b, and c for the entire system where adenine has been removed; (d) dynamics of wave functions a, b, and c for the entire system where adenine is represented only by the partial charges of its atoms; (e) dynamics of wave functions a, b, and c for the entire system where adenine is represented only by the partial charges of the atoms of its NH_2 fragment; (f) dynamics of wave functions a, b, and c for the entire system augmented by the photolyase local atomic charges.

Figure 2a shows the evolution of wave functions a, b, and c in the entire system. The electron excitation starts from the donor, flavin, (initial overlap equal to 1) and reaches the acceptor, cyclobutane dimer, (initial overlap equal to 0). The presence of adenine and the cyclobutane lesion triggers the charge flow from the LUMO+1 and LUMO+2 of flavin to cyclobutane. The LUMO does not participate in the dynamics and is inactive.[12]

LUMO+1 and LUMO+2 have a significant component on the methyl group that points toward the lesion. Analysis of the dynamics therefore suggests that the atomic orbitals of the methyl group need not be populated at time $t = 0$, which also implies that classical hyper-conjugation effects are not important in the transfer.

Figure 2b shows the evolution of orbitals d, e, and f. The absence of the methyl group destroys the transfer, in agreement with previous findings.[12] The methyl group is essential for the passage of the unpaired electron from the cofactor to the dimer. The simplest explanation is a proximity effect (see also below). Cyclobutane hardly receives any charge from the LUMO and LUMO+1 located on $FADH^-$. Some remaining transfer occurs via LUMO+2, which, however, is not involved in the first singlet excited state.

Figure 2c shows the evolution of orbitals a, b, and c in a system where adenine is absent. At apparent odds with the results of ref 12, the absence of adenine strongly affects the transfer. Although not explicitly stated, in the molecular dynamics of ref 12, removal of adenine must have produced a molecular rearrangement that brought other fragments close to the active moieties. The local charges of these fragments may affect the electron dynamics and the transfer. With this notion in mind, it was decided to perform further dynamics of orbitals a, b, and c where the adenine atomic charges were introduced in the total Hamiltonian of the system. Apart from adenine itself, such charges can represent the role played by approaching fragments that move toward the active site when adenine is removed.

Figure 2d shows that the evolution in the presence of the Coulomb charges of the adenine atoms triggers the transfer to the donor from LUMO+1 and LUMO+2. Noteworthy is the periodicity of the oscillation associated with the transfer that increases by a factor of ~2 when compared to the results of Figure 2a. An electrostatic perturbation (included in the Hamiltonian of the system) on the "adenine-side" is therefore necessary to promote the charge flow. The perturbation can be rather small, and, in Figure 2e, is introduced by using only the local atomic charges of the NH_2 fragment of adenine, which suffice to trigger the electron flow. The nitrogen atom charge was −0.807, while the hydrogen atoms charges were +0.342. The maximum transfer is achieved in a time longer than when all adenine charges are present. The presence of the charges of the amino group of adenine suffices to direct the electron flow to the cyclobutane lesion. Figure 2f presents the largest simulation performed in the present work. Orbitals a, b, and c propagate in the entire system that was augmented by the atoms of the photolyase protein described by their charges located at the positions of the crystal structure. The charges are taken from the AMBER model and are to be taken with caution. The presence of these charges can affect the transfer negatively, and only LUMO+2 is active. Because of the limitations and possible inaccuracies of the charges of photolyase, we did not further pursue the issue. The important finding is that the charges present in the environment can hinder or promote the transfer to cyclobutane and therefore healing of the lesion. Electrostatic interactions play a crucial role in promoting or suppressing the ability of the electron to reach the cyclobutane lesion.

In the series of numerical experiments of Figure 2, LUMO+1 of flavin is the most active in promoting the transfer. To simplify

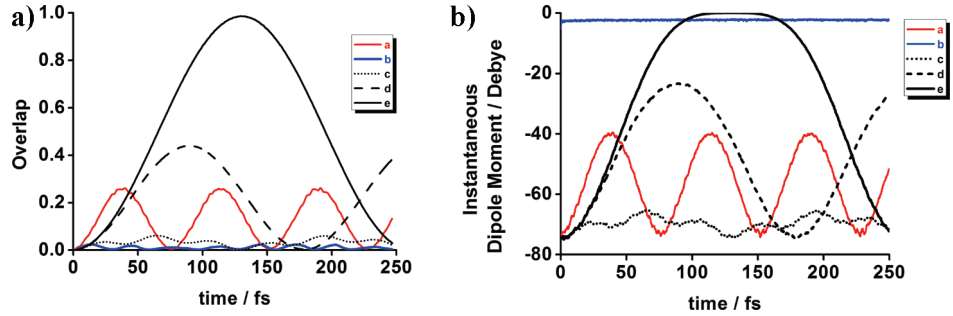

Figure 3. (a) Dynamics of the LUMO+1 transfer to the cyclobutane moiety. (b) Contribution of the LUMO+1 to the instantaneous dipole moment on $FADH^-$. The lettering follows the cases of Figure 2.

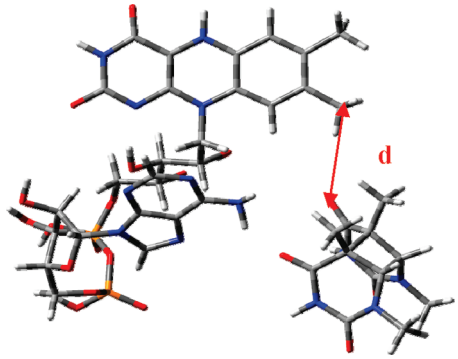

Figure 4. In red, the direction of the distance variation between donor, $FADH^-$, and acceptor, cyclobutane dimer. The red arrow indicates that the methyl group of $FADH^-$ is critical for the ET.

the discussion, we focus on this orbital with the intent of identifying the role of the partial atomic charges in its propagation toward the cyclobutane fragment. Figure 3a summarizes the charge flow to the acceptor under the conditions presented in Figure 2. The maximum transfer is obtained, in the longest time, when only the charges of the NH_2 group of adenine are present. Figure 3b shows the value of the contribution of the LUMO+1 to the instantaneous dipole moment of the donor. To a larger overlap of the propagating wavepacket with the acceptor, i.e., transfer, corresponds a larger dipole moment variation on the donor. Dipole moment variations can be expressed in terms of polarizability of the system. In short, the conclusion is that adenine charges serve to make the propagating wave function polarizable. Other correlations were attempted, but the results of Figure 3a,b provide the simplest explanation for the role of adenine.

In the wavepacket dynamics, the molecular orbitals of Figure 1 at $t = 0$ do not vary when the distance between $FADH^-$ and cyclobutane is modified. They are the unperturbed orbitals. The quantum chemical Hamiltonian, H, that governs the time evolution is, however, a function of the distance between donor and acceptor. Figure 4 shows a simple variation of the distance between donor and acceptor that is taken to assume four values, namely, (a) 3.79 Å, (b) 3.89 Å, (c) 3.99 Å (experimental value), and (d) 4.09 Å.

Figure 5 shows two evolutions of the overlap of the LUMO+1 orbital of Figure 1b as a function of the distance. They correspond to the situations of Figure 2a and Figure 2d, which are taken as illustrative examples of the dynamics. In the entire system (Figure 5a), the largest transfer to the acceptor is found at the longest distance, while the fastest oscillation toward the acceptor is at the shortest distance. In a two-state system, the periodicity of oscillation is inversely proportional to the coupling between the states, while the amount of transfer is related to the energy difference between the interacting states. The two features determine the rate of transfer. Because of the opposite trends with the distance of oscillatory period and total transfer, qualitatively the rate of transfer should be roughly constant over the range explored. During the propagation, external perturbations, such as vibrations, cause the wavepacket to collapse and locate it on cyclobutane (or on the initial moiety). They therefore are critical in determining the observed rates of transfer.

When adenine is described by its charges (Figure 5b), the period of oscillation is nearly constant, and the shorter the distance between donor and acceptor, the larger the transfer and therefore the rate.

Figure 5c summarizes the maximum overlaps observed during the propagation for the two cases. The presence of only the partial local charges of adenine broadens the region of distances where the overlap is large and moves it to lower distances.

Table 1 summarizes the results of the simulations performed as a function of the distance, d. The three cases presented coincide with those of Figure 2 where a sizable transfer takes place.

By inspection and comparison with the figures, one finds:

(1) The transfer is periodic in time, the oscillatory period ranges from ~24 to ~299 fs (these values are twice those required to reach minimum and maximum).
(2) The amount of overlap (transfer) differs from case to case and can be as small as zero or almost equal to one.
(3) The ET varies as a function of the distance.
(4) In the entire system of case a, in the range 3.79−4.29 Å, the maximum transfer is achieved at a distance $d = 4.09$ Å; when adenine is present only in the form of electrostatic interactions (case d), in the range 3.29−4.19 Å, the maximum is shifted to $d = 3.69$ Å; moreover, the time scale of the transfer is proportional to the amount of transfer, if the maximum transfer is small, the period of the transfer is short.
(5) The absence of the methyl group of $FADH^-$ brings the amount of transfer close to zero for all distances.
(6) In all cases, removal of adenine reduces the amount of transfer to the cyclobutane ring.

In agreement with point 6, Prytkova et al.[12] found that the preferential channel for the transfer is via the methyl group of $FADH^-$. Finding 7, however, is in apparent contrast with the results of that work. The authors found that the transfer occurs even in the absence of adenine. They reported results averaged over molecular dynamics runs, which include nuclei displacement and, although not explicitly stated, the rearrangement of photolyase upon removal of this largish moiety. A different result was obtained in refs 10 and 11, where tunneling current calculations showed that the main ET path to the dimer takes place with a through-space jump to the adenine ring and ends

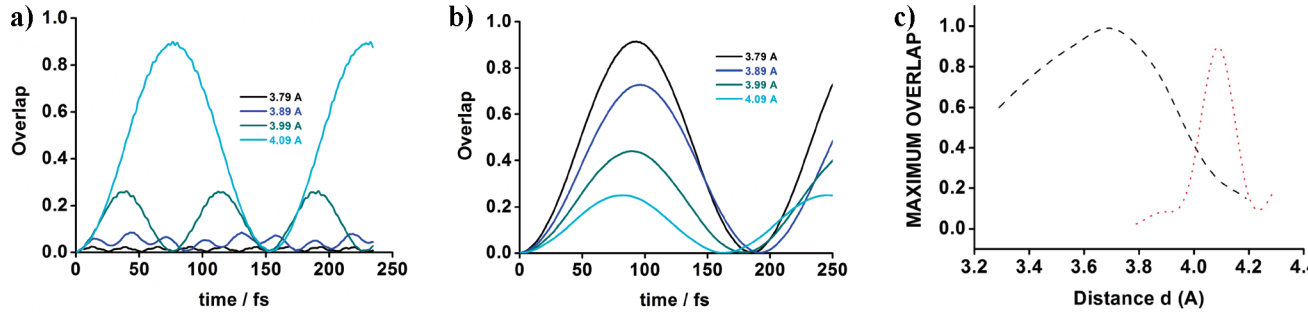

Figure 5. Dynamics of transfer to cyclobutane as a function of the distance: (a) as in the case of Figure 2a; (b) as in the case of Figure 2d; (c) maximum overlap for the cases in a (red curve) and b (black curve).

TABLE 1: For the Cases of Figure 2 when LUMO+1 Transfers the Charge, Time When the Maximum Overlap with Cyclobutane (CB) and the Minimum Overlap with $FADH^-$ Is Reached, Together with the Values of the Overlaps[a]

case from figure 2	time (fs)	CB overlap	$FADH^-$ overlap
a	12.4, 42.9, 39.9, 76.5	0.024, 0.084, 0.261, 0.899	0.975, 0.916, 0.739, 0.100
d	93.5, 96.3, 89.4, 82.6	0.914, 0.728, 0.440, 0.250	0.085, 0.272, 0.559, 0.749
e	85.6, 106.2, 129.5, 148.9	0.849, 0.952, 0.986, 0.850	0.151, 0.048, 0.016, 0.150

[a] The multiple entries (separated by commas) refer to the values for the different distances: 3.79, 3.89, 3.99, and 4.09 Å.

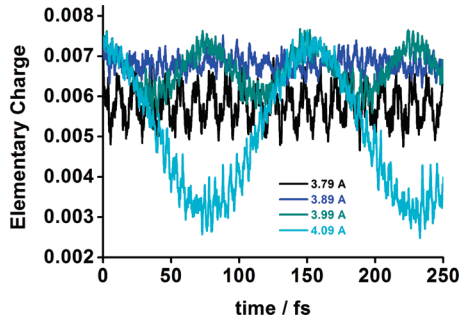

Figure 6. Electronic charge in time of the carbon atom of the methyl group by which the ET occurs.

with a second through-space jump from the adenine to the dimer. The wavepacket dynamics indicates that adenine contributes to the ET by polarizing the orbital. The molecule presence is not necessary *per se*, rather its partial atomic charges are required. Additional simulations show that electrostatic interactions affect the ET and in certain cases, can even inhibit it (case f of Figure 2). Adenine is hardly a simple spectator; its presence provides the necessary electrostatic interactions to promote the transfer and sterically keeps the influence of the surrounding medium and its charges under control.

A final mechanicistic issue concerns the way in which the electron density flows through the methyl group of flavin. It could take place with a constant electron density flowing through it, or, alternatively, with charge density accumulating on it before moving over to the cyclobutane fragment. Figure 6 presents the flux for LUMO+1 in the entire system. The transferring population oscillates in periodic waves. In keeping with the tetravalent nature of the carbon atom of the methyl group, the variation in time of the density is quite modest, although in relative terms it can vary by a factor greater than 2.

Conclusion

In view of the present work, the two existing pictures of direct and indirect ET pathways from photolyases proteins to repair DNA damage induced by UV light appear to be complementary.

The direct pathway, where the electron is transferred from the terminal methyl group of flavin to the carbonyl group of a thymine[8,9] holds even when the charge flux is examined at an atomic level of detail. The indirect pathway, where the electron is ultimately transferred from the amino group of adenine to the carbonyl groups of the thymine dimer,[10,11] has to be partly revised. As emphasized by femtosecond time resolved florescence experiments,[9] the direct role of adenine must be excluded. However, its presence is required to provide extra thrust to the moving charge.

The electron dynamics simulations find that (i) the flow occurs via the terminal methyl group of $FADH^-$, in agreement with the extensive calculations of Prytkova et al., and (ii) the adenine moiety, in general, and its amino group, in particular, are required to polarize the wave function of the donor and drive the transfer toward the cyclobutane lesion.

Since polarization effects can be obtained by the presence of other chemical moieties in the vicinity of the donor and acceptor, it may be possible to exploit this information in the design and development of artificial repair systems.

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