The singlet excited states of the model DNA duplex (dA)10.(dT)10 are studied. Calculations are performed in the exciton theory framework. Molecular dynamics calculations provide the duplex geometry. The dipolar coupling is determined using atomic transition charges. The monomer transition energies are simulated by Gaussian functions resembling the absorption bands of nucleosides in aqueous solutions. Most of the excited states are found to be delocalized over at least two bases and result from the mixing of different monomer states. Their properties are only weakly affected by conformational changes of the double helix. On average, the highest oscillator strength is carried by the upper eigenstates. The duplex absorption spectra are shifted a few nanometers to higher energies with respect to the spectra of noninteracting monomers. The states with larger spatial extent are located close to the maximum of the absorption spectrum.

Introduction

It is well known that absorption of UV light by DNA induces photochemical reactions which may provoke carcinogenic mutations.1-2 The first step of such a series of events leading to alteration of the genetic material is the formation of the so-called Franck-Condor excited states, that is, singlet excited states formed instantaneously upon photon absorption, without any prior relaxation. Their characterization is necessary to understand how electronic excitation energy is transformed into chemical energy within the double helix.

The main issue regarding the singlet excited states of the double helix is whether they are localized on single bases or delocalized over a certain number of them. Very rapidly, the opinion that photons were absorbed by single bases prevailed and guided subsequent photophysical and photochemical investigations involving DNA. This is due to the observation that the DNA UV spectra closely resemble the sum of the spectra of the constituent bases.3 The hypothesis underlying this reasoning is that formation of delocalized excited states should induce large shifts in the absorption spectra. Moreover, a visible splitting of the absorption band around 260 nm was expected.4-5 Although the first theoretical studies that dealt with DNA excitons appeared about forty years ago,6-9 the validity of such statements had not been systematically checked. This contrasts with the sophisticated calculations, which combine quantum chemistry and molecular dynamics, that have been developed recently to describe charge transfer in DNA.8-10

In the framework of exciton theory,11-12 the excited states of a multichromophoric system are linear combinations of the excited states of each monomeric chromophore. Their properties are obtained by diagonalization of the Hamiltonian matrix, in which the diagonal and off-diagonal terms represent the excitation energy of the monomer transitions within the examined system and the electronic coupling, respectively. The delicate point in this type of study is the way that the various terms are calculated. The improvement of computational techniques has occurred during the past decades opened the possibility to determine the exciton-matrix elements with much higher precision compared to earlier studies. New methodologies, introducing quantum chemistry methods in the calculation of the diagonal and off-diagonal terms, were applied in the investigation of various systems such as molecular aggregates and photosynthetic antennas (see for example refs. 13-18). Thus, subtle differences appeared in the properties of various systems and the effect of structural disorder could be evaluated.

Following this progress, it became possible to revisit the DNA excited states, examine the various factors that affect their properties and determine their footprint on the absorption spectra. Within this context, we are interested here in the excited states of the model duplex (dA)10.(dT)10 composed of one strand of adenine bases and one strand of thymine bases. This theoretical study is a continuation of two previous investigations on duplexes consisting of adenine-thymine base pairs19-20 which were performed in parallel with experimental spectroscopic studies.21-22

Our first communication19 focused on the electronic transitions of the monomers that have to be taken into account in the construction of the exciton matrix, and the precision necessary in the calculation of the dipolar coupling. It was shown that it is important to consider the two lowest transitions for adenine, S2→S1 and S0→S2, which can be coupled with the
S₂ → S₁ thymine transition. Moreover, it was demonstrated that the point-dipole approximation, used in previous DNA studies and known to predict artificially large exciton shifts, is not valid for the calculation of dipolar coupling in double helices. It was concluded that dipolar coupling, calculated using atomic transition charges, can induce delocalization of the electronic excitation with double helices having an idealized B-DNA geometry. Our second communication examined how electronic excitation with double helices having an idealized B-atomic transition charges, can induce delocalization of the excitons in the same type of duplexes. It was shown that structural fluctuations reduce the spatial extent of the excited states but excitations still remain delocalized over several bases.

In both of the above-mentioned communications, the hypothesis was made that changes in the internal structure of the monomeric chromophores do not affect the energy of the monomer electronic transitions. Such changes are responsible for the spectral width (homogeneous and inhomogeneous broadening) of the UV absorption bands corresponding to the electronic transitions of monomeric nucleic acids in aqueous solutions. Furthermore, the relative magnitude of the electronic coupling with respect to that of the spectral width is a commonly accepted criterion for formation of localized or delocalized excited states. Dipolar coupling between transitions of nearest bases in double helices amounts to a few hundred wavenumbers, whereas the spectral width of the monomer transitions is about one order of magnitude larger. Accordingly, one would expect complete localization of the excited states. However, this effect may be compensated by the existence of more than one monomer electronic transitions with different polarizations which can be coupled.

The objectives of the present work are twofold. Firstly, it intends to examine how the dispersion of the monomer transition energies, combined with conformational changes, may affect the singlet excited states of (dA)₁₀.(dT)₁₀ related to photon absorption. To this end, the monomer transition energies are simulated by Gaussian functions similar to the corresponding experimental absorption bands. The associated spectral width is supposed to correspond to homogeneous broadening since our previous calculations have shown that the inhomogeneous broadening does not exceed a few wavenumbers (Figure 3 in ref. [20]). Secondly, it aims to establish a correspondence between the absorption spectrum and the properties of the singlet excited states providing some guidelines for experimental photophysical and photochemical studies. In the second section, the methodology followed in the calculation is outlined in a simple way. The results are presented and discussed in the third section. Finally, we summarize our findings and comment on the possible consequences on the photophysics and photochemistry of DNA double helices.

**Methodology**

The duplex excited states were calculated in the framework of the exciton theory. The detailed formalism is described in ref. [19]. The main points of the methodology of the present study are the following:

1. **Duplex Geometry**

   The ground-state conformations used for the calculation of Franck-Condon excited states of (dA)₁₀.(dT)₁₀ were extracted from molecular dynamics simulations which explicitly included solvent molecules and counterions. The calculation procedure is described in ref. [20]. The duplex conformation plays a role in the determination of off-diagonal terms of the exciton matrix because the dipolar coupling between electronic transition moments depends on the angle formed by the corresponding vectors.

2. **Monomer Transitions**

   The lowest transitions for adenine, S₂ → S₁ and S₂ → S₂, which can be coupled with the S₂ → S₁ thymine transition, were taken into account. The oscillator strength and the energy of the maximum associated with those transitions were derived from the experimental spectra of the nucleosides in aqueous solutions, as explained in ref. [19] (Table 1).

<table>
<thead>
<tr>
<th>Transition</th>
<th>Area [/]</th>
<th>Maximum [cm⁻¹]</th>
<th>Width [fwhm/cm⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>adenine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₂ → S₁</td>
<td>0.05</td>
<td>36700</td>
<td>2200</td>
</tr>
<tr>
<td>S₂ → S₂</td>
<td>0.24</td>
<td>38800</td>
<td>3600</td>
</tr>
<tr>
<td>thymine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S₂ → S₁</td>
<td>0.24</td>
<td>37500</td>
<td>4200</td>
</tr>
</tbody>
</table>

3. **Diagonal Terms**

   The excitation energy of each free monomer transition is given by a Gaussian distribution whose width (full-width-at-half-maximum, fwhm) is 2200, 3600 and 4200 cm⁻¹ for the S₂ → S₁ and S₂ → S₂ transitions of adenine and the S₂ → S₁ transition of thymine, respectively (Table 1). In each exciton matrix, a set of monomer transition energies belonging to the above Gaussian functions were considered.

4. **Off-Diagonal Terms**

   The dipolar coupling was calculated using the atomic-transition charge-distribution model. Atomic charges and polarization of the three transitions were derived from quantum chemistry calculations preformed on 9-methyladenine and 1-methylthymine. The coupling corresponding to all the pairs of different bases which formed the duplex was calculated.

5. **Eigenstate Properties**

   Diagonalization of the exciton matrix corresponding to a given duplex conformation and a given distribution of monomer excitation energies yields the k eigenstates of the system [Eq. (1)] which are linear combinations of the wavefunctions |ψₖ⟩ of the...
responding to the monomer transitions:

\[ |k\rangle = \sum_{n=1}^{N} C_{nk} |\Psi_n\rangle \]  

(1)

Since the considered duplex consists of ten adenine bases, each with two transitions, and ten thymine bases, with one transition each, it has thirty eigenstates \(|k\rangle\), whose energies increase from (1) to (30).

**Results and Discussion**

First, we consider the eigenstates of the duplex obtained for one conformation, extracted from molecular dynamics simulations, and a single distribution of monomer energy values chosen randomly. Figure 1 A shows the oscillator strength \(f\) associated with each one of the thirty eigenstates. We observe that some eigenstates are characterized by \(f\) values close to zero and correspond to forbidden electronic transitions. For one third of them, \(f\) is smaller than 0.05, a value corresponding to the weakest monomer transition (S0 → S1 of adenine). Eight eigenstates have an oscillator strength higher than 0.24, the value corresponding to the S0 → S1 transition of thymine and the S0 → S2 transition of adenine (Table 1).

The general trend regarding the difference in the oscillator strength between the monomer and the duplex transitions, discussed above, does not depend on the distribution of the diagonal terms. What varies from one distribution to the other is the precise way in which the oscillator strength is spread over the thirty eigenstates, that is, the eigenstates with low or high \(f\) values. Thus, if we consider the average oscillator strength per eigenstate obtained for 500 sets of free monomer transition energies (Figure 1B), we remark that none of the \(f\) values is zero. However, about 80% of the oscillator strength is concentrated at the upper half of the eigenstates (16 to 30). In Figure 1B, the results obtained for four different conformations of double helix are shown. The total oscillator strength associated with the duplex transitions (5.02) is somewhat lower than the sum of the oscillator strengths corresponding to noninteracting monomers (5.30).

The degree of delocalization of the exciton states is usually quantified by the participation ratio \(PR = 1/L_k\), which represents the number of coherently coupled chromophores \(S_1\) to \(S_2\) if there are more than one electronic transitions per chromophore, \(L_k\) is given by Equation (2):

\[ L_k = \sum_{\text{monomer}\ m} \left( \sum_{\text{state}\ i} \left( C_{ik}^m \right)^2 \right)^{1/2} \]  

(2)

The sum within the square brackets represents the contribution to the eigenstate \(|k\rangle\) of different electronic states belonging to the same monomer (base), for example, the S1 and S2 states of each adenine or the S1 state of thymine.

Figure 2 A shows the participation ratio of the eigenstates obtained for one conformation and a single distribution of monomer excitation energy values. The \(PR\) values are quite spread, ranging from 1 (localization on a single base) to 3.6. The \(PR\) pattern becomes smoother when average values over 500 distributions of diagonal terms are considered (Figure 2B). In the latter case, the values of participation ratio are between

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**Figure 1.** Oscillator strength corresponding to the 30 eigenstates of the duplex (dA)10.(dT)10. A) Single conformation and single distribution of monomer transition energies, both chosen randomly. B) Average values over 500 distributions of monomer transition energies; black, grey, white and dark grey bars correspond to four different conformations extracted from molecular dynamics simulations.

**Figure 2.** Participation ratio corresponding to the 30 eigenstates of the duplex (dA)10.(dT)10. A) Single conformation and single distribution of monomer transition energies, both chosen randomly. B) Average values over 500 distributions of monomer transition energies; black, grey, white and dark grey bars correspond to four different conformations extracted from molecular dynamics simulations.
The spatial extent of a given eigenstate can be illustrated by its energetic topography which is obtained by plotting the 
\((C_{m})^2\) values as a function of the location of each base \(m\) within the double helix. Figure 3 shows the topography of four eigenstates having different participation ratios: 1.0, 1.6, 2.2 and 3.6. They concern the eigenstates \((1)\), \((10)\), \((20)\) and \((24)\) corresponding to the data in Figures 1A and 2A. We observe that each eigenstate exhibits a specific pattern. In the case of \((1)\), 98% of the excitation is borne by the \(S_1\) state of thymine 10. At this point it is important to notice that, in spite of the practically complete localization of \((1)\) on a single thymine chromophore, the associated oscillator strength (0.16) is lower than that of the thymine monomer (0.24). In other words, the existence of delocalized states in the double helix also perturbs the properties of those that remain localized. The eigenstate \((10)\) is mainly built on the \(S_2\) states of adenines 5, 6 and 7; we also note a small participation (about 7%) of the \(S_1\) state corresponding to thymines 3–5. In the case of \((20)\), 77% of the excitation is located on the \(S_1\) state of adenine 4 and the neighboring adenine bases share about 10% each. Finally, three adenine and four thymine bases participate in eigenstate \((24)\). From the topographies in Figure 3, it appears that the various eigenstates, corresponding to the examined energy distribution and configuration, extend over different parts of the double helix. Thus, if internal conversion among the eigenstates (intraband scattering) occurs more rapidly than any other relaxation process, it would result in energy transfer along the double helix (\((24) \rightarrow (1)\)) as well as between the two strands (\((24) \rightarrow (20)\)).

It is difficult to draw a clear limit between localized and delocalized eigenstates. We consider, in an arbitrary way, that an eigenstate is localized if, among the thirty coefficients \((C_{m})^2\)
, there is one whose value is higher than 0.9. In other terms, delocalization occurs if at least 10% of the excitation is shared with at least one other base. Following this definition, 75% of the eigenstates, calculated for 500 distributions of diagonal energies, can be viewed as delocalized although their extent is limited over only very few bases. Moreover, half of the spatially delocalized eigenstates are also electronically delocalized, in the sense that they result from mixing of different monomer states, for example, mixing of the thymine \(S_1\) with the adenine \(S_1\) or the adenine \(S_2\). This happens because the absorption bands associated with the monomer transitions largely overlap.

After having examined the effect of monomer spectral width on the oscillator strength and the spatial extent of the eigenstates corresponding to a single conformation of the duplex, we compare the influence of the conformational changes. Figures 1B and 2B show the \(f\) and PR values per eigenstate obtained for four different conformations. We observe that both properties exhibit only a weak dependence on the geometry adopted by the duplex, which arises from the variation of the off-diagonal terms.

The spatial extent of the duplex excited states may increase by the simultaneous action of coulombic interactions and interactions due to orbital overlap (in particular interchromophore charge transfer). The calculation of this type of interaction is very tedious and has not been achieved so far for DNA double helices. Quantum-chemical calculations performed for stacked aromatic molecules have shown that orbital overlap interactions may be of the order of one hundred wavenumbers. A very rough estimation of the combined action of dipolar coupling and orbital-overlap interactions, can be made by adding a constant term of 100 cm\(^{-1}\) to the off-diagonal terms corresponding to nearest neighbors. For a base \(n\), we considered the bases \(n-1\) and \(n+1\) on the same strand, and the bases \(20-n\), \(21-n\) and \(22-n\) on the opposite strand as nearest neighbors. Figure 4 shows that, as expected, this additional coupling term makes the participation ratio twice as large.

The absorption spectrum corresponding to a given conformation of the duplex is constructed by plotting the oscillator strength of the thirty eigenstates obtained for each one of the 500 sets of monomer transition-energy values. Figure 5 shows the spectrum calculated for a single conformation as well as the average obtained for four different conformations. A nano-
meter scale is used in the plots to make the connection with usual experimental conditions. We observe that, in line with what was found for the oscillator strength and the participation ratio, structural changes have only a weak influence on the spectrum profile.

The simulated duplex spectra, although similar to the experimental spectra, do not intend to strictly reproduce them. As a matter of fact, symmetric Gaussian functions were used to simulate the monomer transition energy, whereas the experimental bands are rather asymmetric. Moreover, at short wavelengths, higher-order transitions overlap with those taken into account in the simulations. Finally, charge-transfer interactions, neglected here, are expected to induce a bathochromic shift of the duplex spectrum as well as a change in the oscillator strength. In spite of the above limitations, the calculated spectra of \((dA)_{10}(dT)_{10}\) allows us to show the effect of the formation of delocalized excited states due to dipolar coupling. To this end, we compare them with the spectrum corresponding to noninteracting monomers, obtained by adding the three Gaussian functions that represent the energy distribution of three monomer transitions, the area under each Gaussian being proportional to the associated oscillator strength. We observe that the duplex spectra are only slightly blue-shifted (7 nm at the maximum) with respect to the spectra of noninteracting monomers and, most importantly, they do not exhibit any apparent splitting.

It is interesting to visualize the position of the thirty eigenstates \((k)\) and their participation ratios on the duplex absorption spectrum. This is shown in Figure 6 where data obtained for a single duplex conformation using 500 sets of the monomer energy distribution, that is, a total of 15000 values, are plotted. Each excited state \((k)\) is represented by a linear segment (Figure 6A). We observe that the positions of the various eigenstates largely overlap. Regarding the dispersion of the participation ratio over the absorption spectrum, we notice that the more extended eigenstates are located close to the absorption maximum (Figure 6B). In contrast, the eigenstates located near the spectral edges are rather localized on single bases. The plots in Figure 6 show that excitation at a given wavelength will populate eigenstates with different indices corresponding to various distributions of monomer energy transitions; their relative proportion depends on the associated oscillator strength. For example, laser excitation at 267 nm, already used for the study of such type of duplexes by femtosecond fluorescence spectroscopy, populates mainly eigen-
states between (10) and (20), which are the most delocalized and result from mixing of different monomer states. In contrast, laser excitation at 295 nm, used to study these compounds by single-photon counting, creates mainly localized excited states.

Summary and Comments

The main results of our theoretical study performed for the model duplex (dA)_{10}·(dT)_{10} can be summarized as follows:

1) The spectral width reduces the spatial extent of the duplex singlet excited states and increases the mixing between different types of monomer excited states (S_1 and S_2 of adenine, S_3 of thymine).

2) Most of the duplex excited states, calculated by taking into account only dipolar coupling, are delocalized over at least two bases. The degree of delocalization increases by the combined action of coulombic and short-range interactions. Short-range interactions associated with interchromophore charge transfer, which have not been precisely calculated so far for double helices, could also be responsible for the well-known DNA hypochromism.

3) The properties of the duplex exciton states, whose calculation is based only on dipolar coupling, are not very sensitive to conformational changes.

4) The only difference between the duplex absorption spectra and those of noninteracting monomers is that the former are only slightly shifted to higher energies. This contrasts with what is commonly accepted, namely, that delocalization of the excitation should induce large spectral shifts and an apparent splitting of the absorption band.

5) Excitation at various parts of the absorption spectrum leads to the formation of excited states with different degree of delocalization: small at the spectral edges, larger near the maximum.

Delocalization of the excitation over pairs of adjacent aminopurines, incorporated in a double-stranded oligonucleotide, has been evidenced recently by fluorescence measurements. Delocalization of the excitation over native bases, even if it is restricted to a short range, is expected to affect both energy transfer and excited-state reactivity. The former was implicitly considered to proceed via a hopping mechanism and thus limited by the extremely short fluorescence lifetimes of nucleic acids. Regarding the latter, one could wonder if, for example, cyclobutane dimer formation is favored or hindered when the energy of one photon is shared by two neighboring thymine bases. These types of questions may inspire experimental studies aiming at the understanding of DNA photodamage within a novel context.

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