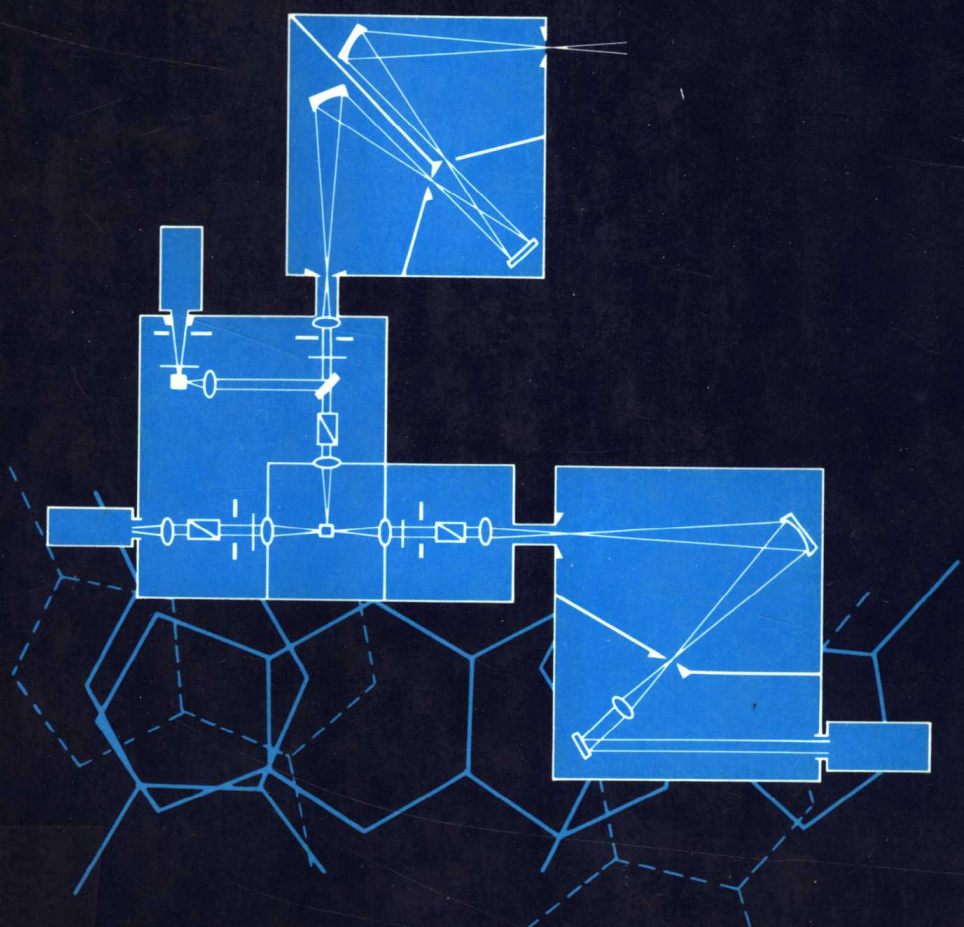
BIOORGANIC PHOTOCHEMISTRY

Volume 1: Photochemistry and the Nucleic Acids



Edited by

Harry Morrison

Bioorganic Photochemistry

Photochemistry and the Nucleic Acids

Volume 1

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Preface

This volume represents the first in a series of monographs which will be devoted to the field of bioorganic photochemistry. The field of bioorganic chemistry has virtually exploded in recent years and it is not surprising to find this development mirrored in the area of photochemistry. Traditionally, photochemical research at the interface of chemistry and biology has been labeled as "photobiology," but the emphasis of significant portions of this literature has increasingly moved toward a merger of biochemical and organic chemical methodologies. Thus the term "bioorganic photochemistry" better reflects the chemical thrust of many of the exciting new developments in such areas as photosynthesis, vision, photoaffinity labeling, and the photochemistry of biologically important molecules such as the proteins and the nucleic acids. In all these cases, chemists are working toward an understanding at the molecular level and employing the latest in synthetic, mechanistic, and spectroscopic chemical techniques.

The nucleic acids, in particular, have fascinated photochemists over the years because of the significance of *in vivo* photomutagenic processes. More recently, we have come to recognize the role of the nucleic acids in drug photoallergy and phototoxicity, become more aware of light-induced protein–nucleic acid cross-linking, and have learned to capitalize on the use of external reagents and light to probe nucleic acid structure. It would therefore seem appropriate that a current survey of the field of bioorganic photochemistry begin with reviews concerning photo-induced modifications of the nucleic acids. It is hoped that the reviews in this monograph will indeed prove useful to readers approaching the topic from a "bio", an "organic," or a "photochemical" perspective.

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West Lafayette, Indiana
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Chapter 1

The Photochemistry of Nucleic Acids

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1 PHOTOPHYSICAL ASPECTS

1.1 Photophysics in the Photochemistry of Nucleic Acids

1.1.1 Aims. Photochemistry is a natural phenomenon for living systems on the earth's surface. Life has developed under continuous sunlight irradiation, this natural light source covering a broad electromagnetic wave spectrum ranging from the infrared (IR) to the ultraviolet (UV). In the UV region, which is the wavelength domain for which the interaction of light with nucleic acid components has been studied almost exclusively, the emission spectrum from the sun is limited in the short-wavelength region below 300 nm due to the ozone layer. Despite this natural wavelength limitation, the sun can be considered as a rather intense UV light source. At midday, with nice weather at 1000 m altitude and assuming the earth's surface to be perpendicular to the incident rays, the electromagnetic energy received by the earth in the range 290 to 330 nm can be estimated to be 10^4 erg cm^{-2} s^{-1} (10 W m^{-2}). By comparison, the mean energy transferred by ionizing radiation at the earth's surface under the same conditions is 10 orders of magnitude lower (Latarjet, 1972). The natural biologic evolution has therefore being conducted under continuous IR, UV, and visible irradiation. A remarkable photobiologic adaptation to solar radiation has resulted which, at the molecular level, has led to the selection of molecules (chromophores) with specific photophysical and photochemical properties.

Nucleic bases are the specific chromophores of nucleic acids in the ultraviolet. Unlike other chromophores involved in several areas of photobiology, such as chlorophyll in photosynthesis or retinaldehyde in vision phenomena, some of the photochemical events that are induced in nucleic acids by UV light give rise to unwanted biological effects at the cellular level. Among these effects are cell killing, photomutagenesis, and photocarcinogenesis. The photosensitivity of the genetic system has been recognized for many years. It is often considered as too high from the biological point of view. As an example, an early report from Setlow (1967) concludes that a bacterium lacking the normal repair mechanism has a 0.5 death probability when only 10 molecular photochemical defects are created per DNA molecule ($\simeq 10^7$ nucleotides). From a molecular point of view, however, it is striking that the quantum yields for the photochemical events encountered in the photochemistry of nucleic acids under physiological conditions are rather low. In fact, it is conceivable that the relative insensitivity of nucleic acid components to photodamages has played an important role in their selection as the primary carriers of the genetic information.

As discussed extensively later in this chapter, the photochemistry of nucleic acids concerns mainly the structural and quantitative determination of the various photomodifications induced by UB light. Within this framework, the aim of photophysics is to provide quantitative knowledge of the excited states produced by the absorption of a photon by a specific DNA component and a detailed description of the various events triggered by this absorption and ending with the creation of a stable photoproduct on the same or on a different DNA

component. Such a description implies the knowledge of several parameters describing the lower energy levels of each of the DNA building blocks. Among them are the energy levels of the singlet (S_1, $S_2 \cdots S_n$) and triplet (T_1, $T_2, \cdots T_n$) excited states and the $^1E(0\text{–}0)$ and $^3E(0\text{–}0)$ values in particular (Fig. 1.1); the lifetimes of the S_1 and T_1 states from which the molecular fluorescence and phosphorescence are emitted, respectively; and the first-order deactivation rate constants of the fluorescence emission (k_f), the radiationless internal conversion process from the S_1 state (k_i), the intersystem crossing to the triplet states (k_{isc}), and their corresponding quantum yields (ϕ_f, ϕ_i, $\phi_{isc}, \ldots$). The main optical spectroscopies used for this purpose are absorption spectroscopy, static and dynamic fluorescence spectroscopies (including static and dynamic anisotropy measurements), phosphorescence spectroscopy, and triplet–triplet ($T_1 \rightarrow T_n$) absorption using flash photolysis methods. In addition, although less common, circular and linear dichroisms, as well as reflection spectra of polarized light oriented along the various axes of single crystals sometimes connected with

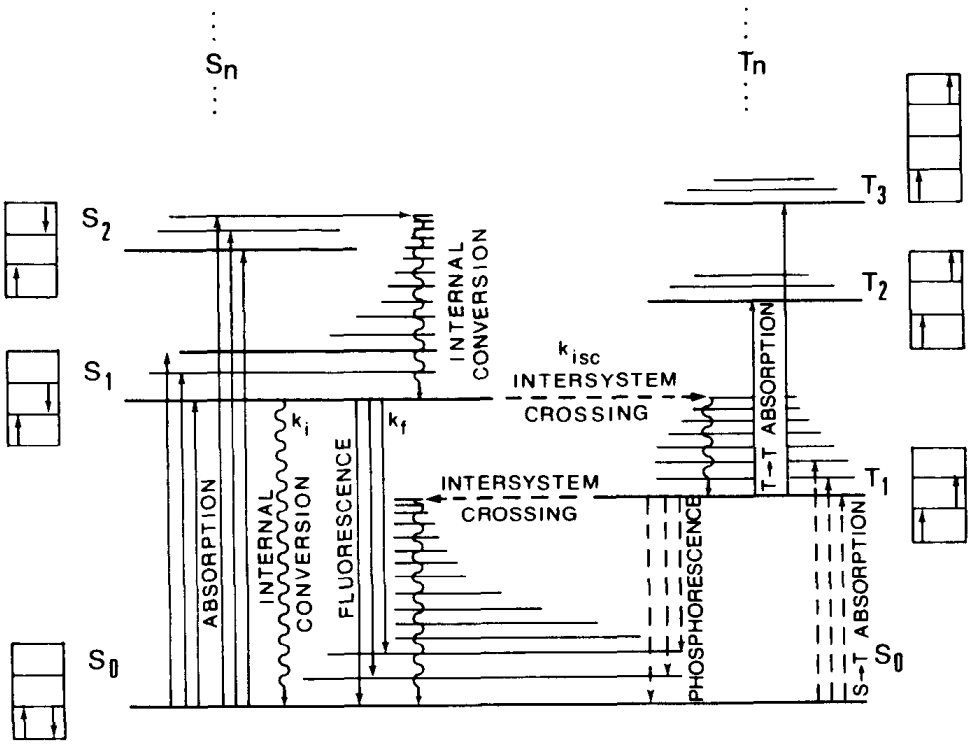


Figure 1.1 Schematic Jablonski diagram indicating the lower energy levels of an organic molecule with an even number of electrons and the various transitions that can occur between these states. Straight lines correspond to radiative transitions, wavy lines to nonradiative ones (internal conversion and intersystem crossing). Vibrational substates of each electronic state are also shown. Times are typical. As seen in the text nucleic base excited state lifetimes differ substantially from typical values (see Section 1.3).

molecular orbital calculations, such as CNDO/S – CI, are useful for the determination of the orientation of the dipole moments of the various absorption transitions.

Although considerable progress has been made in the photophysical area during the last two decades, the picture of the excited state structure and dynamics of nucleic acids remains incomplete and fragmented. This is due to the relatively complex chemical structures of the DNA and RNA components and their tridimensional macromolecular architecture, and to the related experimental and theoretical difficulties encountered in their approach.

1.1.2 Complexity of the Chemical Structure Involved. Compared to the usual objects of study of organic physical chemistry, nucleic acids indeed appear as complex molecules (Saenger, 1983). They are heteropolymers containing at least four different nucleic bases. Adenine (A), guanine (G), thymine (T), and cytosine (C) are involved in the DNA structure, whereas A, G, C, and uracil (U) are the most commonly found bases in RNAs (Fig. 1.2*a*). Within nucleic acids, the hydrogen atoms linked to the nitrogen N(9) of the purine bases (A and G) and to the nitrogen N(1) of the pyrimidine bases (T and C) are substituted by a five-carbon sugar (2-deoxy-D-ribose in DNA, D-ribose in RNA) (see the nucleoside formulas in Fig. 1.2*b*). The complete nucleic acid building blocks further involve the presence of phosphoric acid (see the nucleotide formulas in Fig. 1.2*c*). Among the three characteristic components of nucleotides, the nucleic bases are the only constituent molecules endowed with excited states that can be populated by direct UV irradiation, with maxima of about 250 to 270 nm. Most of the photochemical and photophysical investigations are therefore devoted to effects related to such transitions. Sugar-phosphate chains, as well as nucleic bases, also absorb shorter-wavelength radiation in the vacuum-UV. However, due to experimental limitations in the vacuum-UV area, photochemical research, using these electronic transitions is just beginning to develop (see Section 1.2 for experimental aspects and Section 2.4 for chemical aspects).

When describing electronic transitions in organic molecules, one is naturally tempted to turn to the polycyclic hydrocarbons for which many investigations have been carried out the past several decades. Benzene and naphthalene might be thought to be interesting model compounds to better understand the excited state of pyrimidine (Pyr) and purine (Pur) bases respectively. A rapid examination of the chemical structures shown in Fig. 1.2*a* for the common pyrimidine and purine bases, however, reveals the kinds of problems that have to be faced which are not encountered in polycyclic hydrocarbons:

1. With such a low symmetry, the neat rules that allow us to characterize the molecular orbitals of benzene or naphthalene are of little help in understanding those of pyrimidines or purines.
2. All nucleic bases have more π electrons than centers; consequently they are not isoelectronic with known hydrocarbons, and simple correlations with

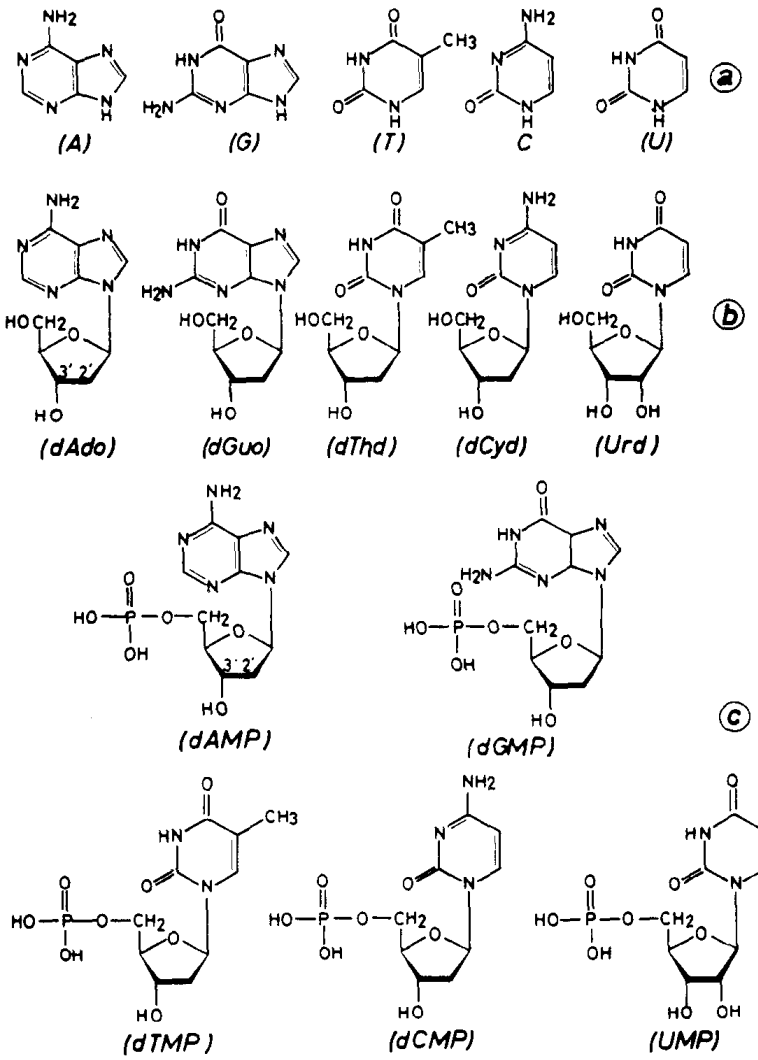


Figure 1.2 Chemical structures of the nucleic acid building blocks. (*a*) Purine bases (Pur): adenine (A), guanine (G); pyrimidine bases (Pyr): thymine (T), cytosine (C), and uracil (U). (*b*) Nucleosides: 2′—deoxyadenosine (dAdo), 2′—deoxyguanosine (dGuo). 2′—deoxythymidine (dThd), 2′—deoxycytidine (dCyd), and uridine (Urd). (*c*) Nucleotides: 2′—deoxyadenosine monophosphate (dAMP), 2′—deoxyguanosine monophosphate (dGMP), 2′—deoxythymidine monophosphate (dTMP), 2′—deoxycytidine monophosphate (dCMP), and uridine monophosphate (UMP).

known model molecules are not allowed. As an example, whereas pyrimidine can be well understood as an inductively perturbed benzene (Moffitt, 1954; Murrell, 1963; Ellis et al., 1972; Scott et al., 1982; Callis et al., 1983), the same cannot be said for Pyr bases. This is probably due to the fact that benzene has six π electrons on six centers, whereas Pyr bases have 10 π

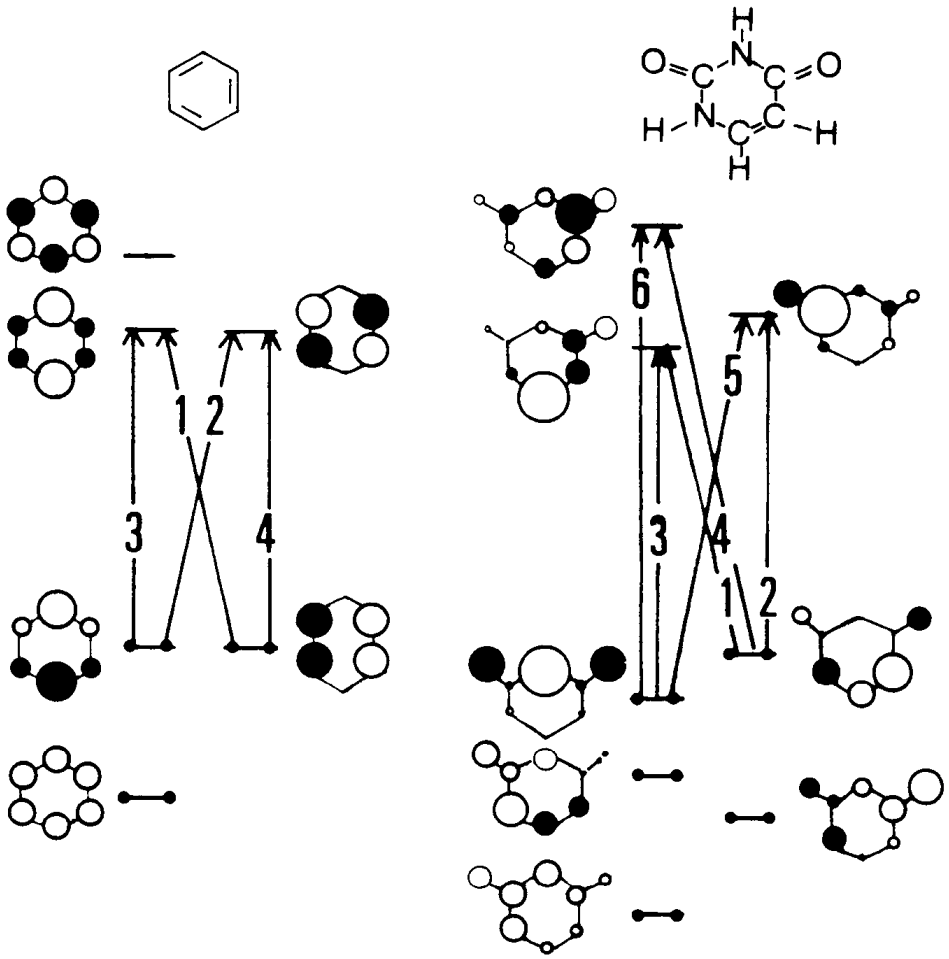
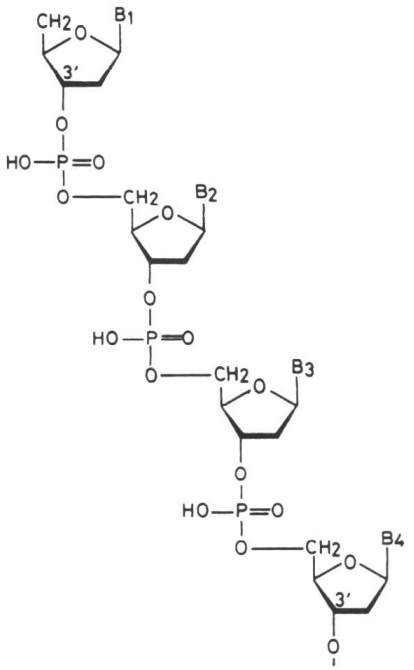


Figure 1.3 Comparison between the molecular orbital diagrams for the π electrons of uracil (right panel) and benzene, as a reference (left panel). Energies and coefficients are from CNDO/S calculations. The energy scales are the same except for shifts to align the highest occupied orbitals. Symmetric molecular orbitals are displayed on the left side of each panel, and antisymmetric ones are displayed on the right side. The diameters of the circles are proportional to the coefficients and black and white indicate the sign. Number on the excitations give the order of configuration [Data redrawn from Callis (1983).]

electrons with eight centers (Sprecher and Johnson, 1977; Callis, 1983). Figure 1.3 illustrates how molecular orbitals and energies of uracil differ from those of benzene.

3. Due to the presence of numerous nonbonded electron pairs, excitation not only of bonding ($\pi \rightarrow \pi^*$) but also of nonbonding ($n \rightarrow \pi^*$) transitions can occur. The few absorption bands observed in the UV are therefore probably envelopes of several electronic transitions that overlap.



(a)

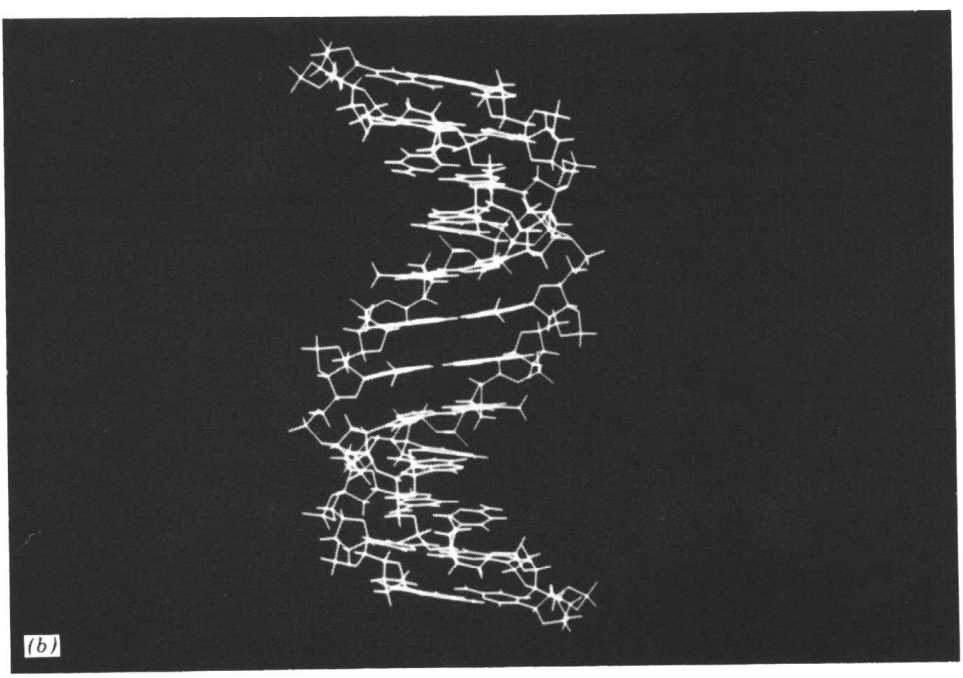


Figure 1.4 Chemical and spatial arrangement of nucleotides for a DNA fragment. (*a*) Phosphodiester linkages joining the 5′-position of the 2′-deoxyribose of one mononucleotide to the 3′position of the other (*b*) Computer drawing of the double-stranded dodecanucleotide d(CGCGATATCGCG)$_2$.

4. Finally, the existence of mobile protons allows for tautomeric equilibria with consequent interferences of the spectroscopic properties of the various species participating in the equilibria.

In addition to the physical problems inherent in the chemical structure of nucleic bases, proper understanding of the photophysics of nucleic acids requires that one takes into account the fact that within DNA and RNA, nucleotides are linked by phosphodiester linkages joining the 5′-position of the D-ribose (or 2-deoxy-D-ribose) of one mononucleotide and the 3′-position of the other (Fig. 1.4*a*). These linkages are responsible for the spatial tridimensional organization of nucleic acids (Fig. 1.4*b*) in which specific hydrogen bonds are formed between bases located at the same level of the two strands and specific stacking interactions take place between adjacent bases. From a spectroscopic point of view, nucleic bases in their excited states can no longer be considered as isolated molecules. The simple molecular deactivation diagram recalled in Fig. 1.1, and the related parameters discussed briefly above, have to be corrected for the fact that within nucleic acids, the chromophores are close enough together to permit electronic interactions. Among the possible consequences are the formation of exciplexes and the possibility for excitation energy to be transferred from one nucleic base to the other one or even to be delocalized over several bases. As for other photophysical phenomena, the formation of exciplex states has been discussed primarily for polycyclic hydrocarbons [see, e.g., Birks (1970)]. Schematically, the formation of an exciplex (or excited complex) corresponds to the formation of a complex between a chromophore in an excited state and another molecule in its ground state. It occurs between two species which have no tendency to form a complex in their ground states, as a result of the completely different molecular behavior of a chromophore in its excited and ground states. The formation of an exciplex is, in particular, mediated by charge-transfer and exchange interactions. The interaction may be quite strong and the chromophores are drawn together until they are closer than in their equilibrium position in the ground state (Fig. 1.5). Due to the fact that the ground state is dissociative at close approach of the two species, the structured emission due to transition between vibronic states is lost in the exciplex. The existence of an exciplex can be detected experimentally in the emission spectrum by the appearance of a broad structureless red-shifted emission, without alteration of the corresponding absorption spectrum. Exciplex- and excimer[†]-type emissions have indeed been detected by emission spectroscopy in many di- and polynucleotides (see Section 1.3) and it is most likely that some of them are the precursors of biologically relevant photoadducts. The other important consequence of electronic interactions between neighboring bases is the possibility of electronic energy transfer between nucleic bases, that is, the special event by which a photon has been

[†] Whereas the term *exciplex* refers to an excited state interaction between two different chromophores, the term *excimer* (excited dimer) applies only to an excited state interaction between identical chromophores.

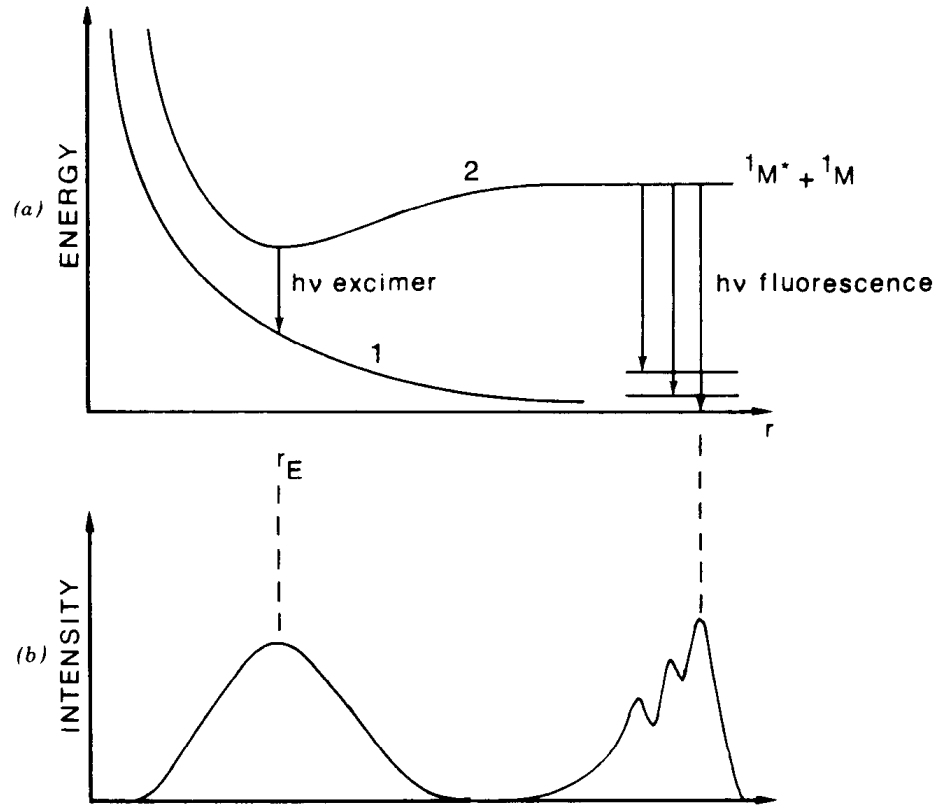


Figure 1.5 Schematic representation of excimer formation and its radiative deactivation. (*a*) Variation of the potential energy of a pair of parallel molecules as a function of their intermolecular separation *r*. Curve 1: the two molecules are in their ground state ^1M and the potential curve is repulsive. Curve 2: one of the molecules is in its first excited singlet state $^1M^*$. The potential curve is the sum of a repulsive term and of a new attractive interaction mediated by charge transfer and exchange interactions. The resulting curve shows a potential well that defines a r_E distance at which the two molecules of the complex are at equilibrium. The right part of the diagram corresponds to the energy diagram of the isolated molecule as defined in Fig. 1.1. (*b*) The corresponding emission spectrum of the isolated molecule (right part) and of the excimer (left part), the latter being considerably red-shifted (lower energy of the transition) and showing no vibrational structure.

absorbed by a given base and the excitation energy transferred—by a more- or-less rapid diffusion process—to another base of same or different chemical nature. In DNA under physiological conditions, this event remains an open question, although it has been observed for some time that the lowest excited T_1 triplet state among the four bases is that of thymine (Section 1.3). This is an important point since energy transfer from higher to lower-lying states is strongly favored and must somehow be related to the fact that thymine is the main photochemical target within DNA. The last-mentioned effect is the

possibility of delocalized electronic states as observed in some periodic structures, such as semiconducting crystals. This phenomenon, although very exciting, has been the object of much speculation. It will not be discussed in this chapter.

1.1.3 Survey of the Evolution of the Research. Several stages can be clearly distinguished in the development of research on excited states of nucleic acids. These steps are separated by marked discontinuities which, themselves, are correlated with significant progress in the spectroscopic techniques of excitation, detection, or data analysis. The successive stages are reviewed briefly here, with the recent significant improvements in related physical methods discussed in (Section 1.2).

A few attempts were made in the 1930s (Euler et al., 1935) and 1940s (Stimson and Reuter, 1941) to observe and analyze the luminescence emission from nucleic acids. These experiments were carried out at a time when the luminescence of highly fluorescent polycyclic hydrocarbons was investigated under various experimental conditions, including *in vivo* conditions (Doniach et al., 1943). The use of quartz optics was, however, not generalized at that time and the excitation was performed with the 366-nm mercury line. If such an excitation agrees well for polycyclic hydrocarbons, it is clear that the results reported in the papers cited above are suspect since nucleic acids are transparent at 366 nm.[†] Probably we should retain from this earliest stage the work carried out on absorption by Mason (1954) as the first significant contribution that really opened the investigations in the field of electronic excited states of purines.

The 1960s appear clearly to be the decade when low-temperature nucleic acid luminescence (fluorescence and phosphorescence) was really evidenced and fully investigated. The first report of phosphorescence from adenine derivatives, DNA, and RNA in glycerol at 77 K was made by Steele and Szent-Györgyi (1957), followed by a work on powders at 77 K (Agroskin et al., 1960) and a detailed study by Bersohn and Isenberg (1964). These initial reports were followed by numerous studies, which allowed us to have a good description of the luminescence properties of nucleic acids at low temperature. Relevant works on this topic have been reported by Callis et al. (1964), Cohen and Goodman (1965), Hélène (1966), Longworth et al. (1966), Guéron et al. (1967), Imakubo (1968) and Hønnas and Steen (1970). The following features have been emphasized. Under these special conditions (77 K, alcohols leading to glassy solutions) Pur and Pyr DNA constituents do fluoresce with detectable fluorescence quantum yields ($\simeq 10^{-2} < \phi f < 1^{-1}$). Pur derivatives also phosphoresce with significant quantum yields ($\phi_p \sim 10^{-2}$), whereas a lack of phosphorescence is generally described for Pyr derivatives and attributed to an absence of intersystem

[†]It should be noted that although it is most probable that luminescence of impurities which did absorb 366-nm light was responsible for the emission reported above, some problems related to emissions of nucleic acids excited at wavelengths higher than 300 nm still remain. Several of us have observed (but never published) unusual emissions for which there might be explanations other than impurities. A recent paper by Daniel et al. (1988) discusses the phosphorescence of adenine derivatives excited under these conditions.