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The Triplet Energy of Thymine in DNA

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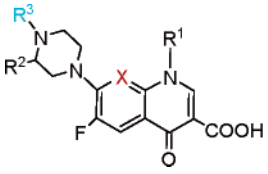
Thymine cyclobutane dimers (T<>T) are among the most important photoproducts formed by direct exposure of DNA to UV radiation and are associated with mutagenic and carcinogenic processes.[1] Besides, this type of damage can be formed upon DNA photosensitization by triplet−triplet energy transfer.[1,2] As the feasibility of this process is linked to the relative excited state energies of the donor and acceptor chromophores, the triplet energy of thymine in DNA is a critical parameter. Its precise value, although not yet definitively established, appears to be markedly different from that of free thymine (Thy) or thymidine (Thd).[2] Moreover, it can be assumed that this is a sequence-sensitive property, as suggested by the site-dependent efficiency of T<>T formation in oligonucleotides.[3] In this context, a triplet energy of 310 kJ/mol has been estimated for Thy and Thd 5′-monophosphate, on the basis of the sensitized generation of a transient T−T absorption at 380 nm in laser flash photolysis experiments.[2] However, compounds with lower-lying triplet states, such as benzophenone derivatives (E_T = 290 kJ/mol) have been shown to photosensitize T<>T formation from Thd, at high nucleoside concentrations, in competition with a more favored Paterno−Büchi photocycloaddition.[4] In DNA, the only approach to address this problem has been the use of photosensitizers of different triplet energies to produce T<>T lesions. In this way, the upper limit for the E_T value of the thymine base in DNA has been progressively shifted from 297 kJ/mol (methoxyacetophenones)[2] down to 290 kJ/mol (benzophenone and phthalimidine derivatives)[4,5] or even lower (fluoroquinolones,[6] E_T between 280 and 260 kJ/mol[7]). Thus, lomefloxacin (LFX), enoxacin (ENX), and norfloxacin (NFX) photosensitize T<>T lesions in DNA, while ofloxacin (OFX) does not. All these compounds share a central heterocyclic skeleton, as shown in Chart 1; the main difference is associated with the nature of X, which can be a carbon atom supporting different electron-donating or -withdrawing substituents, as well as a nitrogen atom.

Chart 1. Structure of Some Fluoroquinolone DNA Photosensitizers



	R^1, X	R^2	R^3
LFX	CH_2CH_3, CF	CH_3	H
ENX	CH_2CH_3, N	H	H
OFX	$CH(CH_3)CH_2OC$	H	H
NFX	CH_2CH_3, CH	H	H
ANFX	CH_2CH_3, CH	H	$COCH_3$

With this background, the aim of the present study was to determine in a more accurate way the triplet energy of thymine in DNA or, more precisely, the minimum value of E_T for a photosensitizer to produce T<>T lesions in DNA. As fluoroquinolones seem to be near to this limit, it appeared reasonable to take them as a starting point.

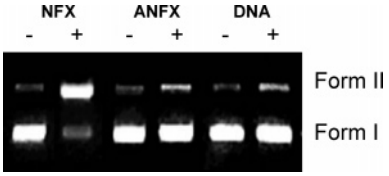


Figure 1. Mixtures containing pBR322 (20 μM in base pair) and NFX, or ANFX (20 μM) were irradiated for 10 min using a multilamp photoreactor (λ between 350 and 400 nm). Endo V treated or not (+/−). Form I and Form II correspond to supercoiled (native form) and circular (relaxed form obtained after SSB formation) DNA, respectively.

The rationale was to find two members of the family with E_T values close to each other, defining a very narrow range comprising the threshold energy capable of triggering T<>T formation in DNA. For this purpose, peripheral substitution (by changing R^3) appeared more convenient than variations in the nature of X at the basic skeleton (Chart 1). Accordingly, NFX and its acetylated derivative ANFX were selected as possible candidates. The former was chosen for convenience, as it is the most simple photosensitizing derivative; besides, N(4′)-acetylation to give ANFX is known to result in a slightly decreased singlet energy, attributed to geometry changes in the excited state associated with N(1′) rehybridization with intramolecular charge transfer.[8] Hence, the E_T of ANFX was also expected to be somewhat lower than that of NFX.

To prove the concept, the DNA photosensitizing properties of the two compounds were investigated first. The experiments were carried out on supercoiled circular DNA (pBR322), which is known to be a very useful tool to detect different types of damages. Conversion of the supercoiled form (also called form I) into the circular form (or form II) indicates single-strand breaks (SSB), which can be observed directly. However, to detect base alterations, DNA repair enzymes have to be used. In this context, T4 endonuclease V has been employed in the present work to reveal T<>T lesions mediated by NFX and ANFX, using photosensitizer concentrations and light doses insufficient to produce direct SSB. Figure 1 shows the obtained results. When DNA was irradiated alone, with or without enzymatic treatment, direct cleavage of DNA did not occur to a significant extent. In agreement with previous observations based on HPLC−MS/MS analysis,[6] NFX (20 μM) was able to photosensitize T<>T formation in DNA, as revealed by irradiation and subsequent incubation with T4 endonuclease V. By contrast, it was remarkable that the closely related N(4′)-acetyl derivative ANFX was inefficient in this assay. Thus, the threshold E_T value for Thy dimerization in DNA seems actually to be in the range defined by the triplet energies of NFX and ANFX. In this context, the main triplet state properties of NFX and ANFX are provided in Table 1 and Figure 2. Laser flash photolysis (LFP) of both compounds in buffered aqueous solutions at pH = 7.4 was

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Table 1. Photophysical Properties of the Triplet Excited States of NFX and ANFX in 1 mM Phosphate Buffer Solution at pH ca. 7.4

parameters	NFX	ANFX
τ_T (μs)	3.5	7
ϵ (M^{-1} cm^{-1})[a]	7900	7800
Φ_T[b]	0.52	0.40
k_q(FBP) (M^{-1} s^{-1})[c]	0.09×10^9	$<0.005 \times 10^9$
k_q(BPC) (M^{-1} s^{-1})[c]	1.5×10^9	0.9×10^9
k_q(NP) (M^{-1} s^{-1})[c]	2.2×10^9	2.1×10^9

[a] The molar absorption coefficients ϵ (M^{-1} cm^{-1}) were calculated for ^{3}NFX at $\lambda_{max} = 620$ nm and for ^{3}ANFX at $\lambda_{max} = 610$ nm by the energy transfer method, using NP as standard. [b] The triplet excited state quantum yield (Φ_T) was determined by the comparative method, using benzophenone as standard. [c] The quenching rate constants were obtained using 0.1−10 mM concentrations of the quenchers in N_2O deaerated medium.

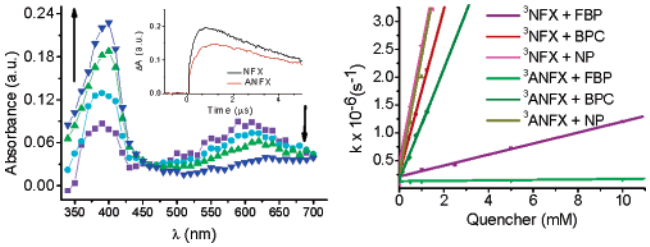

Figure 2. (Left) Transient absorption spectra obtained upon 355 nm excitation of ANFX (0.1 mM) in N_2O-purged buffered aqueous solutions containing BPC (1.25 mM) 50, 170, 300, and 800 ns after the laser pulse. Inset shows the growth profile monitored at 400 nm compared with that obtained for a similar aqueous solution of NFX. (Right) Plot of the NFX and ANFX decay rate constants versus quenchers concentration.

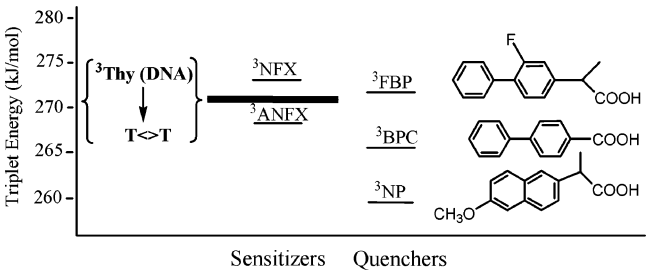

Figure 3. (Left) Triplet energies of the photosensitizers and quenchers used in this work. (Right) structures of FBP, BPC, and NP.

performed upon 355 nm excitation (5 mJ/pulse), both under nitrogen and N_2O atmosphere. Transient T−T absorptions were observed as broad bands with λ_{max} around 600 nm.[9] To rule out the possibility that the failure of ANFX to achieve formation of T<>T was due to a considerable reduction of the intersystem crossing quantum yields (Φ_T), this parameter was determined by the well-established comparative method. The estimated value for ANFX was 0.40, not very different from that found for NFX (0.52).

To determine the E_T of both photosensitizers, energy transfer quenching of the T−T signal by several potential acceptors was studied. The selected compounds were flurbiprofen (FBP), 4-biphenylcarboxylic acid (BPC), and naproxen (NP); they are water-soluble biphenyl or naphthalene derivatives, and their corresponding E_T values are 271,[10] 265,[11] and 259[12] kJ/mol (Figure 3). Thus, LFP of NFX and ANFX was performed at 355 nm in the presence of increasing amounts of FBP, BPC, and NP (Figure 2). The decay of the T−T band was analyzed for both compounds at 590 nm to determine the quenching rate constants. In parallel, the growth of the T−T signals corresponding to the different acceptors at their spectral wavelengths was observed ($\lambda_{max} = 380$ nm for FBP,[10] 410 nm for BPC,[13] and 430 nm for NP[12]). When the rate constants were plotted against the concentrations, six straight lines were obtained (see Figure 2 right). The slopes (intermolecular k_q values) are given in Table 1.

It is generally accepted that the rate constant for energy transfer is nearly diffusion-controlled when the triplet level of the donor is at least 8 kJ/mol above that of the acceptor.[14] With this assumption and using the data of Table 1, the E_T value of NFX and ANFX can be estimated at 273 and 268 kJ/mol, respectively. This leads to ca. 270 kJ/mol as the "functional" value for the triplet energy of Thy in DNA. Obviously, the microenvironments experienced by the different Thy units in DNA are nonequivalent, and therefore, their triplet energies are expectedly different. However, the important information is the E_T required for a given compound to become a potential DNA photosensitizer via T<>T formation (i.e., the functional value). The 270 kJ/mol estimated in the present work constitutes a plausible threshold and provides the basis for an alert rule; any chemical (drugs, cosmetics, pesticides, etc.) with higher E_T has to be considered with regard to its potential photogenotoxicity.

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