Photochemical Cleavage of Phosphodiester Bonds in Oligoribonucleotides†

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ABSTRACT: The release of inorganic phosphate from a variety of mononucleotides and the generation of new phosphomonoester end groups as a result of chain cleavage in a number of oligoribonucleotides have been studied quantitatively as a function of irradiation with 254-nm light. The reaction cross sections for adenosine 5′-phosphate, guanosine 2′(3′)-phosphate, cytidine 5′-phosphate, cytidine 3′-phosphate, cytidine 2′(3′)-phosphate, uridine 5′-phosphate, uridine 2′(3′)-phosphate, dihydrouridine 5′-phosphate, and ribose 5-phosphate are close to 2×10^{-7} m^2/J. The value for UpU is similar. The reaction cross sections, σ, for $(Ap)_n$ where $n = 3$–10 as well as for the oligonucleotides ApUpGp, m^{1}ApCpUpCpGp, CpCpCpCpCpGp, and DpDpDpApApGp increased linearly as a function of the number of phosphodiester bonds and gave values close to 6.4×10^{-7} m^2/J per bond. The cross sections for $(Up)_n$ were also about 6.4×10^{-7} m^2/J per bond for $n = 2$–5 and then, unexpectedly, increased rapidly for $n = 6$–10. By analogy to the carefully studied release of phosphate from ethyl phosphate and several sugar phosphates by 254-nm light [Halmann, M., & Platzner, I. (1965) *J. Chem. Soc.*, 5380–5385], we conclude that the photolysis reactions we have observed are induced by absorption of photons by the sugar phosphate groups rather than the purine or pyrimidine rings. It follows that the quantum yields for chain cleavage of both RNA and DNA have been seriously underestimated since these calculations were based on the assumption that the observed photochemistry is due to absorption of photons by the purine and pyrimidine rings, and the absorption cross section of these rings is roughly 10 000 times greater than that of the sugar phosphate group itself.

It has been known for some time that simple phosphomonoesters undergo photolysis at 254 nm to give inorganic phosphate and a variety of other products (Halmann & Platzner, 1965; Triantaphylides & Halmann, 1975; Trachtman & Halmann, 1977; Triantaphylides & Gerster, 1977). Evidence for a similar reaction with adenosine nucleotides has been published (Goossen & Kloosterboer, 1978). We have extended these studies to a variety of mono- and oligoribonucleotides in order to assess the role that the purine or pyrimidine residues play in this reaction.

The experiments reported in this paper represent the first systematic, quantitative study of phosphodiester bond cleavage produced by irradiation of oligonucleotides with 254-nm light. This work was undertaken to provide information essential for interpreting data we have accumulated on photochemical cleavage of tRNA (Tomasz & Chambers, 1966; I. Kučan et al., unpublished results). Specifically, we needed to know whether random photochemical chain cleavage of oligonucleotides occurs at 254 nm. If it does occur, then we wanted to know the reaction cross section. As we shall show here, "nonspecific" photolysis does occur, but the quantitative results could not have been predicted from studies on simple phosphomonoesters or mononucleotides.

Materials and Methods

With the exceptions given below, all nucleotides [including uridylyl-(3′→5′)-uridine and uridylyl-(3′→5′)-uridylyl-(3′→5′)-uridine] and enzymes were from commercial sources.

Homooligoribonucleotides of the general structure $(Np)_n$, where $n = 3$–9, were prepared by alkaline cleavage of poly(uridylic acid) and poly(adenylic acid) as described by Asteriadis et al. (1976). The cyclic phosphates were opened by incubation at pH 1 for 20 min at 25 °C. The oligoribonucleotides were separated by DEAE-cellulose column chromatography. After separation, each oligonucleotide pool was diluted 3 times with redistilled water and passed through a small (0.3 × 2 cm) DEAE-cellulose column. The adsorbed oligonucleotide was eluted with 2 mL of 1.5 M ammonium bicarbonate, which was then removed by repeated evaporation. The chain length of homooligoribonucleotides was determined from the terminal phosphate to total phosphate ratio after treatment of samples with alkaline phosphatase and with alkaline phosphatase plus snake venom phosphodiesterase, respectively. The structure of the heterooligonucleotides was confirmed by nucleoside analysis by using ultramicro ion-exchange analysis as described previously (Schulman et al., 1973). Dihydrouridylic acid was prepared according to Cerutti et al. (1965); the sample contained about 5% of 5′-uridylic acid.

Irradiation. The radiation source consisted of four Phillips low pressure, 15-W, mercury lamps. The light was filtered through 2 mm of Vycor glass (Corning glass 7905) or, in some experiments, a 20% solution of acetic acid in a 0.5-cm cuvette. Incident flux was determined in the same cuvette in which the compound was irradiated by using uridine 5′-phosphate actinometry as described previously (Schulman et al., 1973). All irradiations were performed in a quartz cuvette, light path 0.5 or 1.0 cm, with constant stirring. The incident flux was about 1.2×10^{17} photons cm^{-2} min^{-1}. The average exposure, $\bar{I}$, was calculated as described by Johns (1969).

Kinetic Measurements. All kinetic measurements were based on inorganic phosphate analysis (Chen et al., 1956). Photolysis of nucleotides was followed by direct analysis of inorganic phosphate released. Photolysis of oligonucleotides was followed by inorganic phosphate analysis after treatment with alkaline phosphatase: Aliquots of irradiated oligonucleotides were digested with alkaline phosphatase in 0.55 M sodium borate buffer, pH 8.5, for 2 h at 37 °C. After incubation, the enzyme was precipitated with HCl at 0 °C and removed by centrifugation. All digestions and assays were performed in siliconized glass test tubes.

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In all cases, the inorganic phosphate released was expressed as a fraction of the starting material. For oligonucleotides terminated with a 3′-phosphate, the inorganic phosphate released by alkaline phosphatase was corrected for that released from the 3′ terminus of the starting material. The molar concentration of new phosphomonoester end groups, P, generated after an average exposure, $\bar{L}$, was calculated from these data.

The molecular cleavage cross sections, σ_m, were obtained from the equation $\log [1 - (P/C_0)] = -\sigma_m \bar{L}/2.303$ (see Results for derivation), where C_0 = molar concentration of starting material, by fitting a straight line to the data by using the least-squares method. Since the actinometry gives photons cm^{-2} min^{-1}, the cross sections were obtained as cm^2/photon. This was converted to m^2/J at 253.7 nm in order to comply with units that are used in the photobiology literature. This conversion, which is commonly made, is not correct unless one has monochromatic light, a condition that is not strictly met here.

Results

Theory. If chain cleavage upon irradiation of an oligonucleotide with 254-nm light is due to absorption of photons by the sugar phosphate moiety and if the reaction cross section/phosphate (σ_p) is the same for each residue, then the cleavage of starting material is given by

$$-dC/d\bar{L} = \sigma_p n_0 C \quad (1)$$

where C = molar concentration of the remaining starting material at an average exposure, $\bar{L}$, and n_0 = number of phosphodiester bonds in the starting material. If σ_p is constant, then the *molecular cross section*, σ_m, is

$$\sigma_m = \sigma_p n_0 \quad (2)$$

σ_m is the probability of cleaving the starting material at any one of its phosphodiester bonds. Combining eq 1 and 2 and integrating give

$$C/C_0 = \exp(-\sigma_m \bar{L}) \quad (3)$$

where C_0 = concentration of starting material at $\bar{L} = 0$.

Chain cleavage produces a new phosphomonoester end group. This can be measured easily and accurately. When $n_0 = 1$, then the initial cleavage products will still contain phosphodiester bonds that can undergo subsequent cleavage to generate new phosphomonoester end groups and complicate interpretation of the kinetics. However, when the concentration of starting material is much greater than the concentration of products, then $C = C_0 - P$ where P = molar concentration of new phosphomonoester end groups. It follows that the new phosphomonoester end groups generated by photolysis of the phosphodiester bond are given by

$$\ln [1 - (P/C_0)] = -\sigma_m \bar{L} \quad (4)$$

The assumptions on which eq 4 is based can be tested experimentally from plots of $\log [1 - (P/C_0)]$ against $\bar{L}$ for oligoribonucleotides of different compositions and chain lengths. For oligonucleotides having the same number of phosphodiester bonds but different compositions, the slopes of the plots should be identical since the slope = $-\sigma_m n_0$. For homooligonucleotides having different numbers of phosphodiester bonds, the slopes should increase linearly as n_0 increases. In order to test these predictions, we have measured the generation of new phosphomonoester end groups produced by irradiation (254 nm) of $(Ap)_{3-10}$, $(Up)_{1-10}$, ApUpGp, m^{1}ApCpUpCpGp, CpCpCpCpCpGp, and DpDpDpApApGp. We have also measured the release of inorganic phosphate from several mononucleotides and from ribose 5′-phosphate.

Table I: Reaction of Cross Sections for 254-nm Photolysis of Some Phosphomonoesters

compounds	$\sigma_m \times 10^7$ (m^2/J)	correlation coefficient
ribose 5-phosphate	2.11 ± 0.05	0.989
A-5′-P	1.92 ± 0 08	0.977
C-5′-P	2.0 ± 0.5	0.968
C-3′-P	1.9 ± 0.5	0.990
C-2′(3′)-P	1.6 ± 0.2	0.998
G-2′(3′)-P	2.2 ± 0.3	0.997
U-5′-P	2.28 ± 0.03	0.995
U-2′(3′)-P	2.0 ± 0.5	0.996
D-5′-P[a]	2.1 ± 0.3	0.989

[a] Dihydrouridine 5′-phosphate.

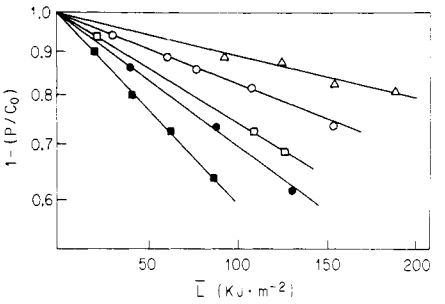


FIGURE 1: Formation of new phosphomonoester end groups by irradiation of oligo(adenylic acids) at 254 nm. (△) $(Ap)_3$; (○) $(Ap)_4$; (□) $(Ap)_5$; (●) $(Ap)_7$; (■) $(Ap)_9$.

Photolysis of Mononucleotides. Dilute solutions of different mononucleotides (10^{-4} M) were irradiated at 254 nm, and the release of inorganic phosphate was followed as a function of exposure. The rate of phosphate release from different nucleotides as well as from ribose 5-phosphate is expressed as the reaction cross section (σ_m), and the data are shown in Table I. The photolysis is very slow and proceeds with about the same cross section for all ribonucleotides studied (average value = 2.0×10^{-7} m^2/J). The nature of the heterocyclic base or the position of the phosphate on ribose (5′, 3′, or 2′) does not influence the photolysis cross section significantly. Even the saturated pyrimidine nucleotide dihydrouridylic acid and the sugar phosphate ribose phosphate undergo photolysis with the same cross section as the nucleotides.

Solutions of deoxynucleoside 5′-phosphate were also irradiated under the same conditions as the ribonucleotides. The reaction cross section for all four deoxynucleotide 5′-phosphates (data not shown) was approximately the same but 3–4 times smaller ($\sigma_{av} = 6.4 \times 10^{-8}$ m^2/J) than for ribonucleotides.

Photolysis of Oligonucleotides. The cross sections for phosphodiester bond cleavage in oligoribonucleotides were determined by measuring new phosphomonoester end groups as a function of average exposure, $\bar{L}$. Representative kinetics for photolysis of oligo(adenylic acids) ranging from 3 to 10 residues in length are shown in Figure 1. The molecular cross sections were calculated from the slopes. The complete results are given in Table II. A plot of σ_m against the number of phosphodiester bonds in the starting material is given in Figure 4. The data fit eq 2 with a coefficient of determination = 0.97. The cross-section/phosphodiester bond, σ_p, obtained from this plot is 6.4×10^{-7} m^2/J.

Similar studies were carried out with several oligoribonucleotides isolated from yeast $tRNA^{Tyr}$. The kinetics are shown in Figure 2. The molecular cross sections, σ_m, are given

Table II: Reaction Cross Sections for 254-nm Photolysis of Some Oligo(adenylic acids)

compounds	$\sigma_m \times 10^6$ (m^2/J)	correlation coefficient
$(Ap)_3$	1.2 ± 0.4	0.941
$(Ap)_4$	1.9 ± 0.3	0.988
$(Ap)_5$	3.2 ± 0.2	0.981
$(Ap)_6$	2.1 ± 0.2	0.987
$(Ap)_7$	3.6 ± 0.4	0.972
$(Ap)_8$	4.2 ± 0.1	0.860
$(Ap)_9$	5.1 ± 0.1	0.986
$(Ap)_{10}$	6.0 ± 0.1	0.977

Table III: Reaction Cross Section for 254-nm Photolysis of Some Oligonucleotides Isolated from Yeast $tRNA^{Tyr}$

compounds	$\sigma_m \times 10^6$ (m^2/J)	correlation coefficient
ApUpGp	1.6 ± 0.1	0.987
m^1ApCpUpCpGp	2.5 ± 0.1	0.991
CpCpCpCpCpGp	3.0 ± 0.9	0.876
DpDpDpApApGp	3.6 ± 0.4	0.970

Table IV: Reaction Cross Sections for 254-nm Photolysis of Some Oligo(uridylic acids)

compounds	$\sigma_m \times 10^6$ (m^2/J)	correlation coefficient
UpU	0.25 ± 0.04	0.927
UpUpU	1.4 ± 0.2	0.998
$(Up)_4$	1.7 ± 0.2	0.990
$(Up)_5$	2.1 ± 0.2	0.979
$(Up)_6$	3.4 ± 0.4	0.979
$(Up)_7$	4.7 ± 0.3	0.989
$(Up)_8$	6.5 ± 0.6	0.981
$(Up)_9$	12 ± 2	0.977
$(Up)_{10}$	27 ± 1	0.998

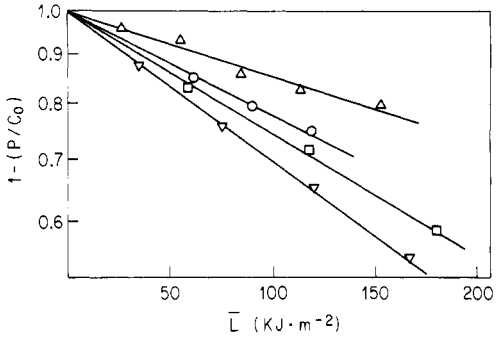


FIGURE 2: Formation of new phosphomonoester end groups by irradiation of some oligonucleotides isolated from yeast $tRNA^{Tyr}$. (△) ApUpGp; (○) m^1ApCpUpCpGp; (□) CpCpCpCpGp; (▽) DpDpDpApApGp.

in Table III, and the data are plotted in Figure 4. They also fit eq 2 and give the same σ_p as the oligo(adenylic acids) (6.4 × 10^{-7} m^2/J).

Photolysis of oligo(uridylic acids) was also studied. The kinetics are shown in Figure 3. The molecular reaction cross sections are given in Table IV and plotted in Figure 4. They increase linearly up to a chain length of five and then deviate rapidly from this linear relationship.

Discussion

The results demonstrate that photolytic release of inorganic phosphate from mononucleotides and phosphodiester bond cleavage in oligoribonucleotides is a general reaction at 254 nm. It is also clear that the primary photochemical reaction

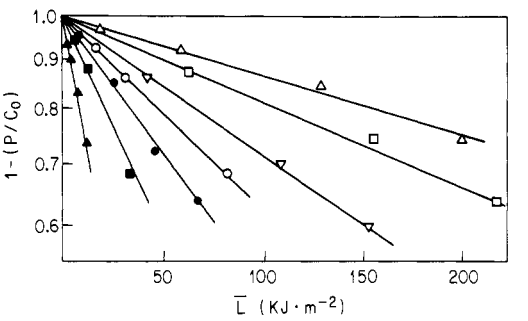


FIGURE 3: Formation of new phosphomonoester end groups by irradiation of oligo(uridylic) acids at 254 nm. (△) UpUpU; (□) $(Up)_5$; (▽) $(Up)_6$; (○) $(Up)_7$; (●) $(Up)_8$; (■) $(Up)_9$; (▲) $(Up)_{10}$.

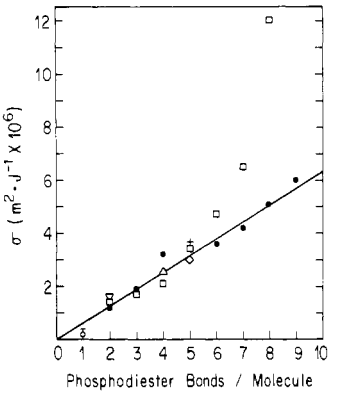


FIGURE 4: Molecular cross sections for photochemical cleavage of phosphodiester bonds in oligonucleotides as a function of chain length. (○) Ribose 5-phosphate, mononucleotides, and UpU; (▽) ApUpGp; (△) m^1ApCpUpCpGp; (◇) CpCpCpCpGp; (+) DpDpDpApApGp; (□) oligo(uridylic acids); (●) oligo(adenylic acids). The line represents a least-squares fit to the $(Ap)_n$ cross sections given in Table II; omitting the $(Ap)_5$ point gives a standard error of estimate = 0.97; slope = 0.64 × 10^{-6} m^2/J.

does not involve purine or pyrimidine photochemistry since the reaction cross sections for a variety of mono- and oligonucleotides are independent of the nature of purine or pyrimidine residues present. For this reason, the reaction is of interest from the standpoint of nucleic acid photochemistry; it is also of potential importance as a factor in the biological effects produced by 254-nm light. Because of this, certain quantitative aspects of the data need further discussion.

It has been known for some time that nucleic acids undergo photolytic cleavage at 254 nm [for a brief review, see Rahn & Patrick (1976) and Gordon et al. (1976)]. Because this has been regarded as a minor photoreaction, it has not received much attention. Part of the reason for this stems from the very low quantum yields that have been reported. For example, the quantum yield for cleavage of tobacco mosaic virus RNA calculated from the disappearance of intact RNA (measured by sedimentation velocity) and the initial absorption of the solution was reported as 10^{-6} (Coahran et al., 1962). More recently a similar calculation for release of inorganic phosphate from AMP using the measured molar extinction coefficient for AMP gave $\phi = 5 \times 10^{-5}$ (Goossen & Kloosterboer, 1978).

Quantum yields are intended to provide a measure for the efficiency with which photons absorbed by a particular chromophore produce reactions. The calculations alluded to above assume that photons absorbed by purines and pyrimidines are responsible for the photolysis of the phosphate bonds. Our results demonstrate that the reaction cross section is the same for release of inorganic phosphate from ribose 5-phosphate, dihydrouridine 5′-phosphate, uridine 5′-phosphate, and

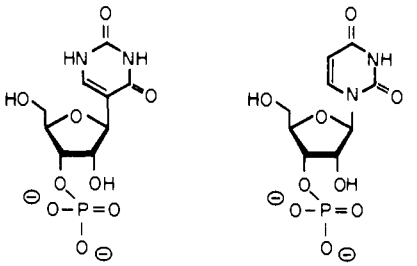
a variety of other mononucleotides. For these compounds the reaction is independent of the purine or pyrimidine rings. It seems probable that the cleavage reaction is due to photons absorbed directly by the sugar phosphate group. Reactions of this type are well documented for simple phosphate esters such as ethyl phosphate (Halmann & Platzner, 1965) and a variety of sugar phosphates (Triantaphylides & Halmann, 1975; Trachtman & Halmann, 1977; Triantaphylides & Gerster, 1977). Thus, one expects nucleotides such as AMP to have two chromophores absorbing photons independently and producing very different photoreactions. The molar extinction coefficients at 254 nm for simple phosphate esters seem to range between 0.1 and 10 (Halmann & Platzner, 1965). The molar extinction coefficients at 254 nm for mononucleotides are about 10^4. Thus, it is very misleading to use the measured molar extinction coefficients of mononucleotides to calculate the quantum yields for phosphate release since most of the absorption observed experimentally is due to a chromophore having nothing to do with the observed photochemistry.

Unfortunately, the molar extinction coefficients for the sugar phosphate groups in nucleotides are not readily accessible. Without these, quantum yields cannot be calculated. However, reaction cross sections can be determined experimentally, and these constants can be used to compare the photolysis of different compounds.[1] For example, the reaction cross sections for ethyl phosphate (calculated from Halmann and Platzner's data) and for AMP (obtained from our data) are 4.9×10^{-9} and 1.9×10^{-7} m^2/J, respectively.[2] Thus, at constant photon fluence, AMP reacts 39 times faster than ethyl phosphate.

The reaction cross section for cleavage of the phosphodiester bond in UpU is the same as that for release of inorganic phosphate from Up or pU ($\sigma_p \approx 2.1 \times 10^{-7}$ m^2/J). This provides further evidence that the photolysis reaction is not dependent on the pyrimidine residue. It also indicates that there is no fundamental difference in the photochemical cleavage of a phosphomonoester and a phosphodiester in these nucleotides. In contrast to this result, the cross section per bond, σ_p, for $(Up)_n$ where $n = 3$–5 is 6.4×10^{-7} m^2/J. This is 3 times the value for pU, Up, or UpU and 130 times the value for ethyl phosphate. Several other oligonucleotides (Table IV) have the same cross section per bond (6.4×10^{-7} m^2/J). These results reinforce our conclusion that cleavage is independent of the nucleotide composition and, therefore, does not involve purine or pyrimidine photochemistry. It also indicates that energy transfer from purine or pyrimidine residues is not involved in the cleavage reaction since the cross section is independent of oligonucleotide composition.

The increase of σ_p for oligonucleotides compared to mononucleotides indicates that the reaction is more complicated than one might expect from simple model compounds. This is particularly evident in the $(Up)_n$ series. As the chain length increases the molecular cross section, σ_m, increases linearly (as expected from eq 2) so the cross section per bond, σ_p, is constant up to $n = 5$. At $n = 5$, σ_p begins to increase rapidly with chain length. For $(U_p)_{10}$, σ_p is 14 times greater than σ_p for Up and 608 times greater than the value for ethyl phosphate: $(Ap)_n$ does not show this effect; σ_p remains constant for $n = 2$–10.

It is not clear whether the effect seen with $(Up)_n$ where $n = 5$–10 is unique; nor is it clear what produces this remarkable increase in σ as the chain length increases. It should be recalled that the reaction cross section for release of inorganic phosphate from pseudouridine 3′-phosphate (**1**) is 3.4×10^{-5} m^2/J (Schulman et al., 1973). This is 160 times greater than the value for uridine 3′-phosphate (**2**). Thus, the oligo(uridylic acid) effect may be due to changes in the configuration of the uracil ring relative to the ribose phosphate as the chain length increases. More experiments are required before further discussion of this is warranted. A study of $(Cp)_n$ would seem to be of particular interest in this regard.



(1) (2)

The results indicate that the true quantum yield for phosphate cleavage is much larger than has been reported previously. For example, if the molar extinction coefficient for the phosphate group in AMP is similar to that in ethyl phosphate (1 L mol^{-1} m^{-1}), then the quantum yield for release of inorganic phosphate is 0.40 based on our data or 0.74 based on Goossen and Kloosterboer's data and not 5×10^{-5} as calculated from the measured extinction coefficient of AMP (Goossen & Kloosterboer, 1978). Similarly, the quantum yield for chain cleavage of tobacco mosaic virus RNA (10^{-6}) based on the measured absorption of the RNA (Coahran et al., 1962) appears to have been grossly underestimated.

Regardless of the quantum yield, which is of interest on photochemical grounds, the results reported here establish that chain cleavage is a general reaction. We have shown with several simple oligonucleotides isolated from $tRNA^{Tyr}$ that the rate at which cleavage occurs is directly proportional to the number of nucleotides in the chain. This experimental verification of eq 1 is important because the equation is based on the assumption that the reaction is random. When this is true then the molecular cross section, which is a measure of the probability for the initial cleavage event, is given by eq 2. While this does seem to be the case for a number of oligonucleotides, it is not true for $(Up)_{6-10}$ where the molecular cross section, σ_m, is much *larger* than one would expect from a random reaction. Additional experiments are required in order to evaluate not only effects of this kind but also ordered structure effects, particularly helix formation, that may alter the cleavage cross section.

We wish to emphasize that one cannot eliminate nonspecific chain cleavage as an important photoreaction simply because the cross section *per bond* is substantially smaller than that for some other photoreaction. To do so ignores the different number of reaction sites that may exist in a nucleic acid for these different reactions. It also ignores the fact that ordered structure does not necessarily affect different photoreactions, or even the same reaction at different places in the nucleic acid,

[1] The reaction cross section is given by the equation $\sigma = \phi\sigma_a$ where σ_a is the absorption cross section. The absorption cross section is a measure of the probability of absorbing a photon; it is related to the molar extinction coefficient by the equation $\sigma_a = 2.303\epsilon \times 10^{-3}$ where ϵ is expressed in L mol^{-1} cm^{-1}. The units for σ are cm^2/μEinstein at 254 nm, which can be converted to m^2/J at 254 nm by the factor 2.1322×10^{-4}; μEinstein = 6.023×10^{17} photons.

[2] The cross section for AMP calculated from the data given by Goossen & Kloosterboer (1978) is 3.6×10^{-7} m^2/J. The difference between this value and the value we have obtained may reflect differences in irradiation conditions (purity of the light, pH, oxygen concentration, etc.).

in the same way. In addition, when biological effects are considered in vivo, other selective effects must be evaluated experimentally since it is well-known that reactions that appear to be kinetically inconsequential can produce amplifiable biological effects. While we make no claim that chain cleavage by 254-nm light is more important than pyrimidine dimer formation, or any other photoreaction, we do believe the reaction deserves further study both chemically and biologically.

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Kinetics for Exchange of Imino Protons in the d(C-G-C-G-A-A-T-T-C-G-C-G) Double Helix and in Two Similar Helices That Contain a G·T Base Pair, d(C-G-T-G-A-A-T-T-C-G-C-G), and an Extra Adenine, d(C-G-C-A-G-A-A-T-T-C-G-C-G)†

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ABSTRACT: The relaxation lifetimes of imino protons from individual base pairs were measured in (I) a perfect helix, d(C-G-C-G-A-A-T-T-C-G-C-G), (II) this helix with a G·C base pair replaced with a G·T base pair, d(C-G-T-G-A-A-T-T-C-G-C-G), and (III) the perfect helix with an extra adenine base in a mismatch, d(C-G-C-A-G-A-A-T-T-C-G-C-G). The lifetimes were measured by saturation recovery proton nuclear magnetic resonance experiments performed on the imino protons of these duplexes. The measured lifetimes of the imino protons were shown to correspond to chemical exchange lifetimes at higher temperatures and spin–lattice relaxation times at lower temperatures. Comparison of the lifetimes in these duplexes showed that the destabilizing effect of the G·T base pair in II affected the opening rate of only the nearest-neighbor base pairs. For helix III, the extra adenine affected the opening rates of all the base pairs in the helix and thus was a larger perturbation for opening of the base pairs than the G·T base pair. The temperature dependence of the exchange rates of the imino proton in the perfect helix gives values of 14–15 kcal/mol for activation energies of A·T imino protons. These relaxation rates were shown to correspond to exchange involving individual base pair opening in this helix, which means that one base-paired imino proton can exchange independent of the others. For the other two helices that contain perturbations, much larger activation energies for exchange of the imino protons were found, indicating that a cooperative transition involving exchange of at least several base pairs was the exchange mechanism of the imino protons. The effects of a perturbation in a helix on the exchange rates and the mechanisms for exchange of imino protons from oligonucleotide helices are discussed.

Relaxation rates of the base-paired imino protons have been measured by proton nuclear magnetic resonance (NMR) experiments in several nucleic acid systems (Crothers et al., 1974; Johnston & Redfield, 1977, 1978; Hurd & Reid, 1980; Early et al., 1981a,b). We recently studied the kinetics for exchange of imino protons in a DNA, RNA, and hybrid oligonucleotide helix (Pardi & Tinoco, 1982). The saturation recovery technique developed by Redfield (Johnston & Redfield, 1977) was used in these studies, and the theory for interpretation of the exchange behavior of imino protons measured by NMR has been discussed by Johnston & Redfield (1981) and Pardi & Tinoco (1982).

The three helices used in this work, the 12-mer [helix I = d(C-G-C-G-A-A-T-T-C-G-C-G)], the 12-mer G·T [helix II = d(C-G-T-G-A-A-T-T-C-G-C-G)], and the 13-mer [helix III = d(C-G-C-A-G-A-A-T-T-C-G-C-G)], have been studied

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