Near Threshold Photo-Oxidation of Dinucleotides Containing Purines upon 266 nm Nanosecond Laser Excitation. The Role of Base Stacking, Conformation, and Sequence†

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The 266 nm nanosecond laser photoionization (PI) of a wide range of dinucleotides containing purines, and related nucleosides and nucleotides, occurs through a combination of one- and two-photon pathways that can be considered as a near threshold PI process. The net PI yields of these DNA and RNA model compounds are of the order of 10^{-2}, whereas the monophotonic component is of the order of 10^{-3}. These yields increase with an increase in the initial ground-state concentration, which is interpreted in terms of a stabilization of their ionization potentials (IPs) due to an increase in the electronic coupling between the bases as the base stacking of the dinucleotides increases on going from 1 to 100 μM concentrations. Photodestruction yields for dinucleotides in the range 10^{-3} to 10^{-2} were determined using low-intensity 254 nm irradiation; these increased in the following order: GpC ≈ TpdG > CpG > ApG. Competition experiments using electron scavengers showed that the participation of the oxidized base in the photodestruction mechanism is 85% for GpC, 64% for TpdG, 59% for ApG, and 18% for CpG. The photoionization yields, estimated from the effect of electron scavengers on the photodestruction yield at low-intensity 254 nm irradiation, correlated well with the monophotonic PI yields of the dinucleotides examined. Differences in base stacking, conformation, and structural fluctuations of the various dinucleotides isomers are proposed to explain the sequence effect observed in the PI yields and photoreactivity at 254 nm. A linear correlation between the net PI yield of various dinucleotides and the calculated IPs reported by Saito and Rösch groups was obtained [Saito, I.; Nakamura, T.; Nakatani, K.; Yoshioka, Y.; Yamaguchi, K.; Sugiyama, H. *J. Am. Chem. Soc.* **1998**, *120*, 12686−12687; Voityuk, A. A.; Jortner, J.; Bixon, M.; Rösch, N. *Chem. Phys. Lett.* **2000**, 324, 430−434]. Altogether, the results strongly suggest the use of near threshold PI yields of small oligonucleotides to estimate the effect of such properties as base stacking, sequence and conformation, base pairing, structural fluctuation, and solvation on the efficiency of hole-trapping and charge transport in DNA.

Introduction

It is well-known that the purine and pyrimidine bases are the DNA and RNA components most sensitive to UV radiation-induced modification or destruction.[1] Damage to these components can occur under oxidative stress conditions such as the presence of oxidizing agents, ionization radiation, photoirradiation with endogenous photosensitizers, and high-intensity laser excitations.[2−5] Since the 1990s, long-range oxidative damage to DNA caused by one-electron oxidation has attracted considerable interest primarily because the suggested key role played by oxidation in aging, mutagenesis and carcinogenesis.[2,4−6]

It is now satisfactorily documented that exposure of DNA and related model compounds to high-intensity laser excitation can be used as an effective and convenient way to generate radical cations of the DNA bases and to promote charge transfer within DNA.[4] Because guanine exhibits the lowest ionization potential among DNA bases,[7] a radical cation injected into duplex DNA can migrate several base pairs to ultimately end up at guanine residues.[2a,8] Saito and co-workers have shown experimentally and by ab initio calculations at the HF level of theory that in the one-electron oxidation of B-form DNA, the 5′-G residues of 5′-GG-3′ steps, act as better traps of hole migration across the DNA π stack.[9]

Several charge transport mechanisms have been proposed to account for a wide range of distance dependence and efficiency of hole migration observed in duplex DNA. The most likely mechanism involves a combination of superexchange transfer over a few base pairs and the transport of the charge over long distances by a hopping mechanism.[5f,g,10] Evidence has been presented for the preponderant role played by such factors as IPs,[9,11] base sequence and stacking,[5d,12] dynamical fluctuations of the DNA structure,[13] and the electronic coupling[14] and energy gap[15] between the bases on the electron-transfer and hole-trapping and -injection efficiency in DNA.

Various groups have used theoretically calculated ionization potentials (IPs) for XGY (X, G, Y = A, C, G, T) triplets to predict the reactivity of different sites in DNA toward one-electron oxidation and to estimate energies for hole-transfer duplexes.[9b,11b] These IPs are in good agreement with experimentally observed relative reactivities of duplexes toward

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† Abbreviations: UV, ultraviolet, HPLC, high performance liquid chromatography; Ade, adenine; Ado, adenosine; Guo, guanosine; Gua, guanine; dApT, 2′-deoxyadenylyl-(3′→5′)-thymidine; TpdG, thymidinylyl-(3′→5′)-deoxyguanosine; dApdA, 2′-deoxyadenylyl-(3′→ 5′)-2′-deoxyadenosine; GpA, guanylyl-(3′→5′)-adenosine; ApG, adenylyl-(3′→5′)-guanosine; TpdA, thymidinylyl-(3′→5′)-2′deoxyadenosine; GpC, guanylyl-(3′→5′)-cytidine; CpG, cytidylyl-(3′→5′)-guanosine; CMP, cytidine 5′-monophosphate; Cyd, cytidine; Cyt, cytosine; CpA, cytidinylyl-(3′→5′)-adenosine; GMP, guanosine 5′-monophosphate; AMP, adenosine 5′-monophosphate.

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photoinduced one-electron oxidation.[9b,11a] Subsequently, it was shown that stabilization of B^+ in 5′-XBY-3′ triads is significantly influenced by the subsequent base Y whereas the effect of the preceding base X is rather small.[11b] Thus, the estimation of the effect of the sequence, conformation, stacking, and dynamical fluctuations of the bases on the base's IPs are of fundamental importance in understanding the control and efficiency of charge transport and to propose a phenomenological description of a long-range charge transport mechanism in DNA. However, studies on the effect and relevance of such properties on the IPs of the DNA bases are scarcely reported.[9,11,13−15,16]

The dinucleotides can be considered as the simplest model for one-electron photooxidation of DNA biopolymers because their photochemical properties can be understood in terms of the known behavior of the monomeric constituents,[17−22] and because these molecules exhibit a considerable degree of base stacking in aqueous solution at 298 K[23] characteristic of DNA macromolecules. Candeias and Steenken studied the rates of charge transfer of various dinucleotides containing purines in aqueous solutions and observed that hole migration toward adenine and guanine from the pyrimidine base radical cations occurs with a rate constant $\geq 2 \times 10^5$ s^{-1}.[24] In the case of charge transfer in ApG, a rate constant of 2×10^7 s^{-1} was reported. Thus, it was shown that the one-electron oxidation of the pyrimidine bases occurs through a rapid and efficient charge-transfer process toward adenine and from there on to guanine.[24]

Recently, our group and others have shown that 266 nm nanosecond laser excitation of guanine and adenine derivatives in aqueous solution promotes their near threshold PI through a combination of one- and two-photon pathways.[19−21,25] Additional support for this near threshold PI of the purine bases has been obtained by semiempirical results demonstrating that the IP thresholds of the adenine and guanine nucleotides and of the pGpAp dinucleotide in aqueous solutions are 4.9 ± 0.5, 4.7 ± 0.5, and 4.2 ± 0.5 eV, respectively, very close to the one-photon energy at 266 nm (4.66 eV).[16b,26] Because the purine bases are known to have the lowest IPs among the nucleobases (vide supra),[7] the stacking interaction of the dinucleotides containing purines could further enhance the stabilization of their IPs. In this work, we show, for the first time, that the PI of various DNA and RNA dinucleotides containing purines occurs through a combination of one- and two-photon pathways, thus strongly suggesting that near threshold PI of these molecules are obtained at the experimental conditions used.

A direct correlation between the calculated IPs of B-form DNA triplets reported recently by Saito[9b] and Rösch[11b] and the net PI yields obtained for a wide set of dinucleotides containing purines was observed. Thus, it was considered of great interest to investigate if the PI yields of these DNA model compounds could be used to predict the observed hole-trapping efficiency for various B-form oligonucleotides.[9b,11b] A major aim of this work was to determine if the sequence, conformation, and the degree of base stacking of various dinucleotides isomers (ApG/GpA, CpG/GpC, and dApT/TpdA) affect the PI yields (which are proposed to be related to their relative oxidation potentials) and their photoreactivity upon one-electron photooxidation. These properties are likely to affect the control and efficiency of long-range DNA oxidation and thus some preliminary correlations are presented. The dinucleotide sequence isomers differ only in the esterification of the phosphate group to the ribose moieties[21a] and are, thus, simple enough for initial studies of the conformation and sequence effects on the IPs of the DNA bases.

Materials and Methods

The purine, pyrimidine, and dinucleotides used in this work (from Sigma Chemical Co., St. Louis, MO) were of the highest purity available; Tl_2SO_4 (Acros Organics, New York), nitrogen (General Gases and Supplies Co., San Juan PR), and oxyclear disposable gas purifier (Aldrich Chemical Co. Inc., Milwaukee, WI) were used as received. All the solutions were prepared with Nanopure water. The solutions were deoxygenated with ultrapure nitrogen before (15 min) and during the experiments. Unbuffered solutions were used for all the dinucleositides and related nucleosides and nucleotides. The absorption of the irradiated solutions at 266 nm was not allowed to decrease more than 10% as a result of photodegradation during laser excitation.

The PI experiments were done using a laser kinetic spectrometer consisting of a Nd: YAG Q-switched Surelite II Laser from Continuum delivering up to 80 mJ (measured at the position of the optical cell using a Newport Multi-function Optical Meter). The beam profile was Gaussian, unfocused, circular with 0.8 cm diameter at the sample position, and the pulse duration was 11 ns. Time-resolved absorption measurements were made perpendicular to the excitation beam by using a conventional Xe 150 W arc lamp, a monochromator (Spex 1681) and an R928 Hamamatsu photomultiplier tube. Hydrated electron decay signals were recorded with a Le Croy Digital Oscilloscope (Model 9360). Data acquisition and analysis were conducted with a National Instrument Lab View program as previously reported.[27]

Using laser photoexcitation, the formation of the hydrated electron was studied by measuring the transient absorbance near the maximum of its absorption band at 700 nm. The absorption coefficient at this wavelength is 18 700 M^{-1} cm^{-1}.[28] The PI quantum yields were determined relative to the hydrated electron yields obtained using ultrapure nitrogen-saturated solutions of KI (0.16 M) as an actinometer.[29] The initial absorbance of all the dinucleotides and related nucleotides and nucleosides solutions was 0.90 ± 0.02 at 266 nm in a 1 cm optical path unless otherwise stated. The experiments were performed at room temperature (23 ± 2 °C). The net PI quantum yield for the molecules examined, defined as the arithmetic sum of a one-photon and a consecutive two-photon processes, was determined as a function of the laser intensity at 266 nm from the least-squares slope of a graph of the absorbance at 700 nm. In the experiments, the electron absorbences were measured both as a function of increasing and decreasing laser intensity. This procedure was followed to avoid errors due to fluctuations in the laser intensity. The results in both experiments were almost identical. Each point on the graph represents the average of 10 laser shots, and the PI values are the average of two measurements.

Laser excitation at 266 nm of a cuvette containing Nanopure water did not produce transient absorptions at 700 nm, corroborating that the observed transient absorptions result from excitation of the bases. Nikogosyan et al.[30] have reported that at irradiation intensities of $(2-3) \times 10^{13}$ W/m^2 the ionization and/or photodissociation of water is not significant in aqueous dilute solutions of the bases. In our experiments, the maximum excitation intensity used was approximately 2×10^{11} W/m^2. Thus, a two-photon PI of water can be disregarded as contributing to the observed hydrated electron signal.

Sample preparation, irradiation conditions, and procedures for quantum yield determination in the continuous 254 nm irradiations have been previously described.[18,19]

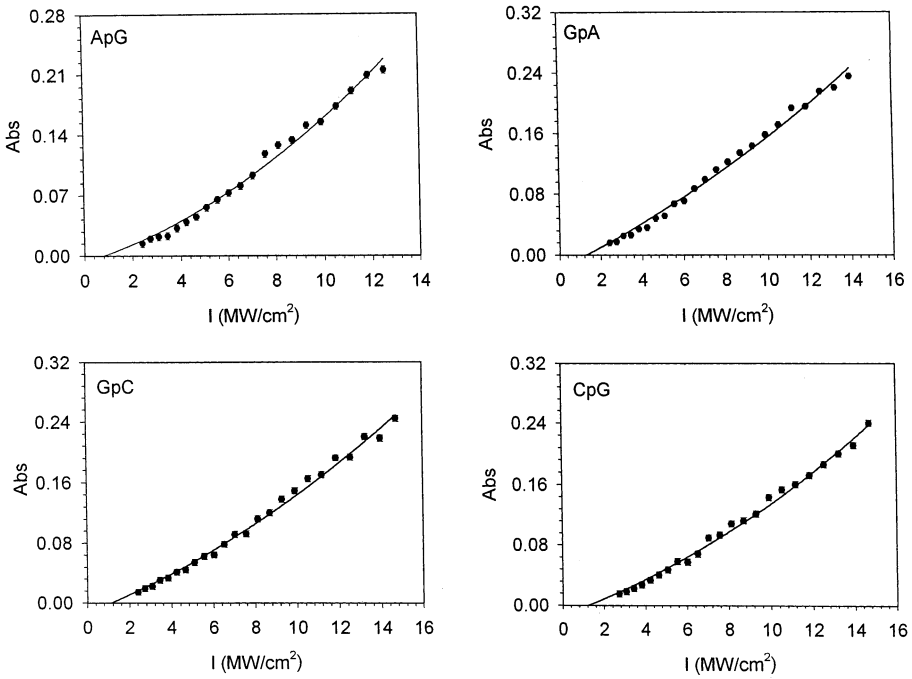

Figure 1. Initial hydrated electron absorbance at 700 nm as a function of the laser intensity (λ_{exc} = 266 nm, 11 ns pulses) for ApG/GpA and CpG/GpC sequence isomers.

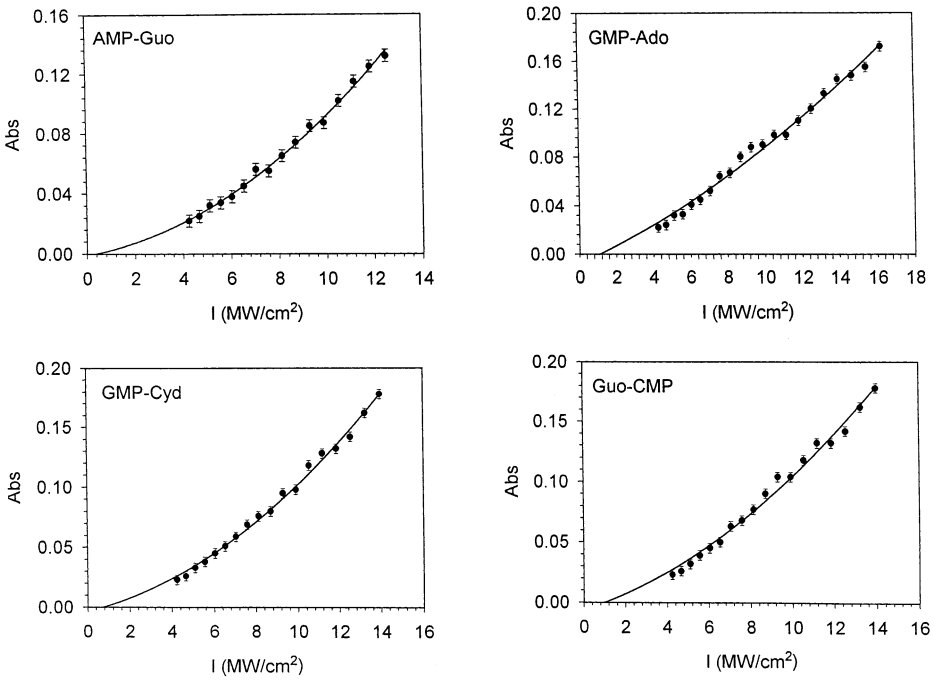

Figure 2. Initial hydrated electron absorbance at 700 nm as a function of the laser intensity (λ_{exc} = 266 nm, 11 ns pulses) for an equimolar mixture of nucleosides and nucleotides.

Results

Near Threshold One-Electron Photooxidation of DNA and RNA Model Compounds in Unbuffered Aqueous Solutions upon 266 nm Nanosecond Laser Excitation. Studies of near threshold PI of DNA model compounds in aqueous solution are of fundamental importance for understanding the influence of the electronic properties of the DNA bases on a variety of processes such as one-electron oxidation by high-intensity laser irradiation, ionizing radiation, charge-transfer processes, and mechanisms of mutagenesis and carcinogenesis.[2−6] Typical plots of the initial absorbance at 700 nm after the laser pulse for the dinucleotides and related nucleotides and nucleosides examined in this work are presented in Figures 1 and 2. For all the molecules studied, a quasi-linear relationship was observed, implying the saturation of the intermediate state involved in the net PI pathways of the bases.[31] The net PI quantum yields of the DNA and RNA model compounds were measured at 5 and 10 MW/cm^2 to compare these with previously reported values (Tables 1 and 2).[21] At a laser intensity of 10 MW/cm^2, it was observed (Table 1) that for the dinucleotides containing Ade, the net PI yields decreased as follows: dApdA > dApT ≈ GpA ≈ ApG > TpdA > CpA. The PI yield for those containing Gua decreased as GpG > TpdG> GpA ≈ ApG ≈ GpC > CpG, whereas those containing cytosine showed the

TABLE 1: Net PI Quantum Yields for Various Purine-Containing Dinucleotides at Different Concentrations

system	ϕ (±0.004)[a]	ϕ (±0.004)[b]
dApT (abs = 0.90)	0.032	0.036
GpG (abs = 0.90)	0.039	0.043
TpdG (abs = 0.90)	0.027	0.037
dApdA (abs = 0.90)	0.038	0.041
GpA (abs = 0.90)	0.028	0.035
GpA (abs = 0.40)	0.021	0.030
GpA (abs = 0.10)	0.018	0.026
ApG (abs = 1.20)	0.031	0.045
ApG (abs = 0.90)	0.028	0.036
ApG (abs = 0.60)	0.028	0.033
ApG (abs = 0.10)	0.019	0.030
TpdA (abs = 0.90)	0.024	0.030
GpC (abs = 1.00)	0.034	0.037
GpC (abs = 0.90)	0.032	0.035
GpC (abs = 0.60)	0.026	0.031
GpC (abs = 0.30)	0.025	0.031
GpC (abs = 0.10)	0.023	0.030
CpG (abs = 1.00)	0.026	0.032
CpG (abs = 0.90)	0.025	0.031
CpG (abs = 0.60)	0.027	0.030
CpG (abs = 0.30)	0.020	0.029
CpG (abs = 0.10)	0.021	0.024
CpA (abs = 0.90)	0.017	0.027

[a] Measured at 5 MW/cm^2. [b] Measured at 10 MW/cm^2.

TABLE 2: Net PI Quantum Yields for Various Purine Derivatives at Different Concentrations

system	ϕ (±0.004)[b]	ϕ (±0.004)[c]
AMP (abs = 1.14)	0.015	0.017
AMP (abs = 0.90)[a]	0.015	0.021
AMP (abs = 0.57)	0.015	0.019
AMP (abs = 0.13)	0.015	0.021
ADP (abs = 0.90)[a]	0.014	0.025
dGMP (abs = 0.90)[a]	0.020	0.025
GMP (abs = 1.20)	0.023	0.029
GMP (abs = 0.90)[a]	0.015	0.018
GMP (abs = 0.60)	0.023	0.028
GMP (abs = 0.10)	0.025	0.028
dAdo (abs = 0.90)[a]	0.013	0.019
dAMP (abs = 0.90)[a]	0.016	0.020
Ado (abs = 1.18)	0.010	0.017
Ado (abs = 0.90)[a]	0.013	0.019
ATP (abs = 0.15)	0.016	0.019
ATP (abs = 0.90)[a]	0.012	0.020
Ade (abs = 0.90)[a]	0.012	0.017
Guo (abs = 1.20)	0.011	0.016
Guo (abs = 0.90)[a]	0.013	0.017
Guo (abs = 0.48)	0.026	0.032
Guo (abs = 0.32)	0.024	0.029
Guo (abs = 0.11)	0.020	0.024
dGuo (abs = 1.10)	0.023	0.023
dGuo (abs = 0.90)[a]	0.013	0.017
dGuo (abs = 0.57)	0.019	0.023
dGuo (abs = 0.13)	0.019	0.022
9Et−Gua (abs = 1.20)	0.016	0.029
9Et−Gua (abs = 0.90)[a]	0.011	0.016
9Et−Gua (abs = 0.60)	0.022	0.024
9Et−Gua (abs = 0.10)	0.018	0.020

[a] Taken from ref 21. [b] Measured at 5 MW/cm^2. [c] Measured at 10 MW/cm^2.

lowest net PI yields and decreased in the following order: GpC > CpG > CpA. Also, in the case of the dinucleotides containing thymine, significant differences were observed in the net PI yields. For instance, a change of dAdo for dGuo in the thymine dinucleotide had an appreciable effect on the net PI yields; TpdG (0.037) > TpdA (0.030), as expected from the oxidation potentials of the purine bases.[7] In addition, the calculated net PI yields for the isomers dApT/TpdA and GpC/CpG were

TABLE 3: Net PI Quantum Yields for Various Pyrimidines at Different Concentrations and Equimolar Mixtures of Nucleosides and Nucleotides

system	ϕ (±0.004)[c]	ϕ (±0.004)[d]
KI (0.16 M) (720 nm)[a]	0.360	0.360
Thd (abs = 1.16)	0.028	0.031
Thd (abs = 0.90)[b]	0.017	0.020
Thd (abs = 0.10)	0.017	0.022
CMP (abs = 0.90)[b]	0.018	0.025
CMP (abs = 0.60)	0.018	0.020
CMP (abs = 0.14)	0.017	0.017
Thy (abs = 0.90)[b]	0.014	0.019
Cyd (abs = 0.90)[b]	0.015	0.016
Cyt (abs = 1.14)	0.015	0.017
Cyt (abs = 0.90)[b]	0.015	0.016
Cyt (abs = 0.11)	0.015	0.022
CMP−Guo (abs = 0.90)	0.017	0.024
GMP−Cyt (abs = 0.90)	0.016	0.023
GMP−Ado (abs = 0.90)	0.016	0.020
AMP−Guo (abs = 0.90)	0.015	0.021

[a] Taken from ref 28. [b] Taken from ref 21. [c] Measured at 5 MW/cm^2. [d] Measured at 10 MW/cm^2.

different, suggesting an influence of the dinucleotide's sequence and conformation on the electronic properties of the DNA bases. Altogether, the PI results suggest that the IPs of the purine bases depend on the base sequence under the experimental conditions used in this work. The trends of the net PI yields at 5 MW/cm^2 were very similar to those obtained at 10 MW/cm^2 and are in good agreement with those reported previously (Table 1).[21]

The net PI yields of the DNA and RNA model compounds were also measured at different ground-state concentrations (1−100 μM). These results are included in Tables 1−3. For the majority of the monomers, no change in the PI yield with an increase in concentration was observed within experimental errors. However, for GpC, CpG, ApG, and GpA an increase in concentration in the range 4−80 μM resulted in a systematic increase in the net PI quantum yields.

Recent results from our group[19−21] and by LeBreton and coworkers[25] have shown that near threshold PI of DNA and RNA monomers can be achieved through a combination of one- and two-photon pathways at 266 nm wavelength. Thus, it was considered important to establish if one-photon PI pathway was also observed in the high-intensity laser excitation of the dinucleotides containing purines. The initial electron absorbance as a function of the laser intensity was carefully analyzed using different procedures. log−log plots of the data presented in Figures 1 and 2 yield slopes with values in the range 0.96−1.5 (data not shown), due to a mixture of linear and nonlinear pathways in the PI of the DNA and RNA bases. A similar behavior was observed for various purine and pyrimidine derivatives in aqueous solution.[20,21]

The experimental data were further analyzed using eq 1, which has been used to test the presence of a mono- and biphotonic PI pathways:[20,21,32]

$$A/I = a + bI \qquad (1)$$

where I is the laser intensity, a is a coefficient that depends on the quantum yield of the one-photon process, and b is a factor that depends on the molar absorption coefficients and yields of the intermediate species in the consecutive two-photon process.[32] A mixture of one- and two-photon pathways in the formation of the hydrated electrons of the dinucleotides was observed (note the nonzero intercept, Figure 3). Similar results were obtained for equimolar mixtures of the related nucleotides and nucleosides (data not shown).

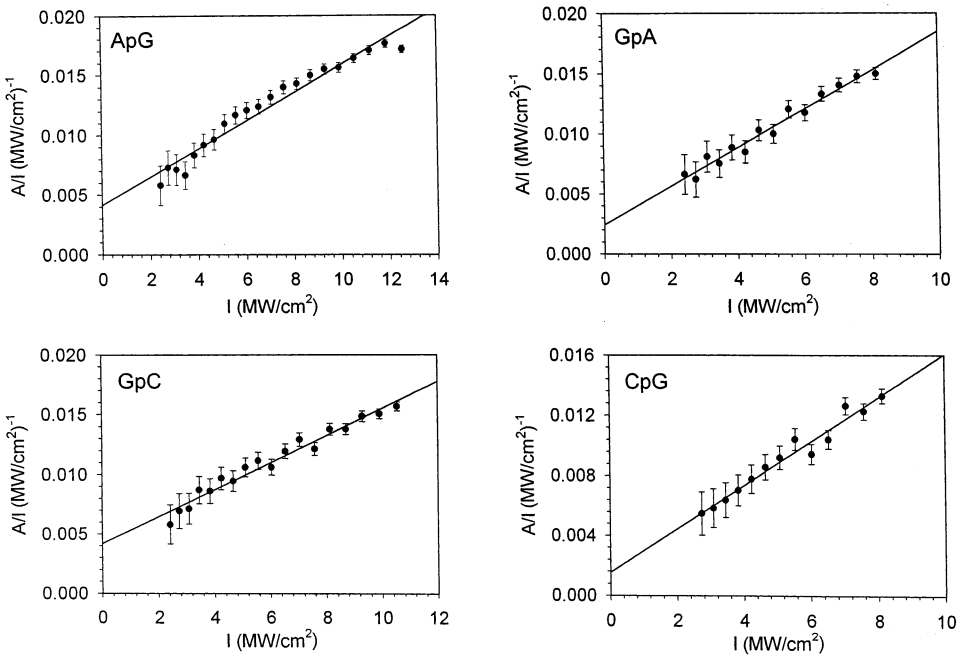

Figure 3. Initial absorbance of the hydrated electron divided by the pulse intensity (A/I) as a function of the laser intensity for ApG/GpA and CpG/GpC sequence isomers.

TABLE 4: Photodestruction Quantum Yields[a] for Various Dinucleotides in Aqueous Solutions at 298K

dinucleotide (75 μM)[b]	φ (photodestruction) $\times 10^2$
ApG[c]	0.51 ± 0.04
ApG, Tl^+ [c]	0.30 ± 0.05
TpdG[c]	2.2 ± 0.2
TpdG, Tl^+ [c]	1.4 ± 0.2
CpG	1.63 ± 0.01
CpG, Tl^+	0.29 ± 0.05
GpC	2.6 ± 0.5
GpC, Tl^+	2.2 ± 0.1

[a] Average of three independent measurements. [b] The concentration of Tl_2SO_4 was 1 mM. [c] Taken from ref 19; see also ref 49.

TABLE 5: Monophotonic PI (φ) of Dinucleotides and Related Nucleotides and Nucleosides

system[a]	φ (266 nm) $\times 10^3$
dApT	8.2 ± 0.2
TpdA	8.0 ± 0.2
GpG	11 ± 2
GpC	9.2 ± 0.8
ApG	9 ± 1
CpA	8 ± 2
CpG	7.1 ± 0.6
TpdG	5.5 ± 0.9
GpA	4.9 ± 1
dApdA	4 ± 3
GMP−Cyt	6.4 ± 0.7
AMP−Guo	5.1 ± 0.6
GMP−Ado	2.9 ± 0.8
CMP−Guo	1.2 ± 0.6

[a] The absorbance at 266 nm was 0.9 ± 0.2.

As far as we know, the presence of a monophotonic component in the PI pathway of dinucleotides containing purine are presented for the first time. These results are in agreement with those obtained recently for various purine and pyrimidine derivatives,[20,21] and with the semiempirical ionization threshold in the range 4.5 ± 0.5 to 5.5 ± 0.5 eV, estimated recently for the nucleotides in aqueous solution.[26] Indeed, for the dinucleotide pGpAp, the calculated semiempirical ionization threshold in aqueous solution is 4.2 ± 0.5 eV,[16c] very close to the energy of one 266 nm photon (4.66 eV).

For almost all the DNA and RNA model compounds, a monophotonic quantum yield of the order of 10^{-3} was calculated from the intercept of plots similar to Figure 3 (Table 5). The values included in Table 5 for the DNA and RNA model compounds correspond to a 15−25% of the net PI yield at 266 nm. In previous studies on the PI of various DNA and RNA polymers, no attempts were made to observe if a one-photon PI component was present in the data.[30a,33] This may be understood from the small but significant contribution of this component to the net PI observed in the current study.

Evaluation of the PI Yields of the Dinucleotides Containing Purines upon Low-Intensity 254 nm Irradiation through the Measurement of Their Photodestruction Yield with and without Addition of an Electron Scavenger. To further assess and strengthen the support for the proposed near threshold PI of the dinucleotides with a photon energy of approximately 4.7 eV, the PI of various dinucleotides using 254 nm low-intensity irradiation were studied. The quantum yields of photodestruction for TpdG, CpG, GpC, and ApG in aqueous solution under anaerobic conditions and in the presence or absence of Tl^+, an efficient electron scavenger,[18−20] were determined and are included in Table 4. We had previously shown that from a variety of electron scavengers, i.e., O_2, Cd^{2+}, Co^{2+}, N_2O, Ag^+, and Tl^+, only the Ag^+ and Tl^+ ions or their reduced forms, do not participate in secondary reactions with the bases or their intermediates.[18−20] In these experiments a concentration of 1 mM Tl^+ was used to scavenge the hydrated electron ($k = 5.4 \times 10^{10}$ M^{-1} s^{-1})[34] at a rate 100 times faster than that of the dinucleotides. At this concentration the absorbance of Tl^+ was less than 0.04 at 254 nm. Thus, under the assumption that the observed decrease in the quantum yield of photodestruction in the presence of the electron scavenger can be related to the electron yield at the low-intensity 254 nm continuous irradiation, the estimated PI yields of TpdG, ApG, CpG, and GpC were $(8 \pm 2) \times 10^{-3}$, $(2.1 \pm 0.9) \times 10^{-3}$, $(13.4 \pm 0.9) \times 10^{-3}$, and $(4 \pm 2) \times 10^{-3}$, respectively. These values are in very good agreement with the monophotonic yields obtained for these dinucleotides using high-intensity laser excitation at 266 nm

(Table 5), providing further support for the proposed near threshold PI of these molecules in aqueous solutions at 266 nm.

Finally, the quantum yield of photodestruction for the dinucleotides studied decreased as follows: GpC ≈ TpdG > CpG > ApG. This result correlates well with the degree of base stacking of these molecules: GpC > CpG > ApG.[23a] The estimated extent of participation of the radical cation (oxidized base) in the photodestruction of the dinucleotides was calculated to be CpG (18%) < ApG (59%) < TpdG (64%) < GpC (85%), Table 4. Thus for almost all the dinucleotides tested, the participation of the oxidized bases in their photodestruction is higher than 50%. These results are in very good agreement with the participation of one-electron oxidation of the bases in isolated DNA,[35] further supporting the low-intensity 254 nm PI of these dinucleotides containing purines. Indeed, very recently, the ionization threshold wavelength of deoxyguanosine (dGuo) in aqueous solution at pH 6.3 has been estimated to be in the range 260 ± 16 nm,[36] lower in energy than a 254 nm photon.

Discussion

Effect of Base Stacking, Conformation, and Sequence on the Net PI Yields of the Dinucleotides. Our results strongly indicate that a near threshold PI of the dinucleotides, through a combination of one- and two-photon components, is achieved upon 266 nm laser excitation. Support for this near threshold PI is provided by the electron photoejection mechanism previously proposed for a wide range of DNA bases and derivatives,[18−20,25] by the estimated monophotonic PI yields obtained from scavenging experiments using 254 nm low-intensity irradiation, which are in very good agreement with the monophotonic yields obtained at 266 nm, and by theoretical calculations demonstrating that the ionization potentials thresholds for various nucleotides[26] and dinucleotides[16c] in aqueous solutions are very close to a 266 nm photon energy. The fact that the near threshold PIs of the dinucleotides were much lower than the gas-phase IPs of the nucleotides[7] shows the preponderant role of water solvation in the stabilization of the excited states of the DNA bases.

The observed trends on the net PI yields for the dinucleotides (Table 1) are interpreted in terms of a reduction of the IPs due to differences in the degree of base stacking interactions in these molecules, i.e., to differences in the stacking association constants. It is proposed that the higher the degree of base stacking interactions, the more efficient is the electronic coupling of the stacked bases in a given dinucleotide. This increase in the electronic coupling of stacked bases will cause a decrease in their IPs, which are directly related to the PI yields. The increase in the net PI yield of the dinucleotides (Table 1) with an increase in concentration is explained in terms of the stabilization of the IPs of the bases. This hypothesis is supported by the fact that a modest, but observable, increase in the one-photon PI yield of the dinucleotides with an increase in their ground-state concentration was observed (data not shown) and by the observation that at a given concentration the one-photon PI yields of the dinucleotides were higher than those of the corresponding monomers (see Table 5, and Table 3 in ref 21).

As the degree of base stacking increases, due to an increase in the concentration of the dinucleotides, an increase in structural rigidity is expected. This increase in rigidity will increase the electronic coupling between the stacked bases, further lowering their IPs and resulting in an increase in the PI yields. An evident support for this proposal is that the observed trends on the net PI yields for the dinucleotides (Table 1) correlate well with the postulated degree of base stacking for various dinucleotides (dApdA > GpA > CpA > ApG)[23] and with the general increase in base stacking:[37] pyrimidine−pyrimidine < pyrimidine−purine < purine−purine. Furthermore, the available association constants for CpA, GpA, and ApA at 20 °C showed that there is a correlation between the degree of base stacking and the PI yields of these dinucleotides.[23b,c] In addition, several groups have presented theoretical evidence suggesting that stacking interactions between the DNA bases reduce the base IPs[9b,16] and theoretical calculations[9b] have shown the IP of the dinucleotides increases in the following order: CpG < GpG < GpA = ApG < GpC, in partial agreement with our experimental observations (Table 1).

The conformation of the dinucleotide has an important effect on the stabilization of the IPs of these DNA model compounds (Tables 1, 4, and 5). This was demonstrated by the fact that the net and monophotonic PI yields for the dinucleotides, Tables 1 and 5, respectively, were higher than those of the individual bases (Tables 2 and 3) or of the equimolar mixtures of the corresponding monomers (Tables 3). This result suggested that not only the stacking interactions but the dinucleotide conformations affect the electronic coupling and the PI yields. Similar conformational effects were observed in the results of the theoretical calculations by Sevilla and co-workers.[16a] They demonstrated that the adiabatic ionization potential of the stacked AT and GC base pairs decreases when compared to those of the individual bases or their base pair configurations in aqueous solutions. Furthermore, theoretical calculations[9b] on GpG have shown that the HOMO molecular orbital can be localized with different proportions (extent) on each base depending on their overlapping area (conformation) and that the first IP of this dinucleotide decreases when compared to an unstacked molecule.

Angelov et al.[4b] have recently shown that oxidation products such as 8-oxo-7,8-dihydro 2′-deoxyguanosine and 8-oxo-7,8-dihydro 2′-deoxyadenosine are generated from the corresponding 2′-deoxynucleosides in a yield that is about 2 orders of magnitude lower than in DNA. This increase in the yield of the oxidation products was attributed to the predominant influence of the DNA conformation and base stacking properties on the hydroxylation reaction of the C8 position of both purine moieties. Our results suggest that this increase can be explained by a decrease in the IPs (or oxidation potential) of the purine bases as compared to the free deoxynucleosides. This can be accounted for in terms of a higher degree of base stacking interactions in DNA, which reduce the structural fluctuations in this macromolecule. This reduction in structural fluctuations results in an increase in the electronic coupling of the bases, which is expected to increase the charge transport efficiency to the more easily oxidized deoxynucleotide in DNA.

The effect of sequence, base stacking, and conformation on the photochemistry of GpC and CpG at 254 nm were also investigated. In this work, we report a significant difference in the photodestruction quantum yield at 254 nm, $(2.6 \pm 0.5) \times 10^{-2}$ for GpC and $(1.63 \pm 0.01) \times 10^{-2}$ for CpG. Moreover, the participation of the oxidized base in the photodestruction mechanism of CpG is only 18%, whereas for GpC it is 75%, a striking difference. These results can be taken as additional evidence for the major role played by sequence, base stacking, and conformation on the photoreactivity and on the IPs of these dinucleotides. It is well-known that these sequence isomers present different degrees of stacking and variations in their conformations.[23a,b] For example, sequence effects have been observed for these and other isomer dinucleotides from their

TABLE 6: Comparison between the Average IP Obtained for Various B-Form DNA Triads[a] with the Net PI Yield of the Related Dinucleotides at a Laser Intensity of 10 MW/cm^2

system	⟨X⟩BY[b]	PI (±0.004)
XGG	7.320	0.043
XAA	7.885	0.041
XTG	8.653	0.037
XAT	8.039	0.036
XAG	7.775	0.035
XCG	8.891	0.031
XTA	8.820	0.030
XCA	9.057	0.027

[a] Taken from ref 11b. [b] These values represent the average IP value obtained from Table 2 in ref 11b for the corresponding triads, X = A, C, G, and T.

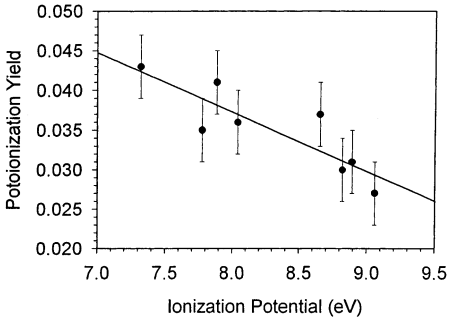

Figure 4. Correlation plot of the dinucleotides (BY) near threshold photoionization yields versus the average relatives IPs[11b] of B-form DNA triads.

proton magnetic resonance spectra,[23d] vacuum UV circular dichroism bands,[38] and optical rotatory dispersion spectra.[39]

The marked difference in photoreactivity among these sequence isomers deserves further consideration. It has been suggested that proton-transfer (PT) reactions may play a prominent role in the fixation of the radical sites.[40] These protonation/deprotonation processes may have drastic effects on the chemical reactivity of the species involved.[22b,41] Thus, it is possible that the higher degree of stacking in GpC than in CpG allows for a fast irreversible PT reaction, resulting in the observed increase of photodestruction of the former. A similar conclusion was reached recently by Sevilla and co-workers.[42] They showed that proton transfer in $GC^{\bullet-}$ and $GC^{\bullet+}$ slows electron and hole transfer in various polynucleotides and in DNA.[42] In DNA, where the bases occur in pairs characterized by a preset hydrogen bonding interaction, the PT reactions are more probable. Because PT along the interbase hydrogen bonds involves only a slight displacement of the equilibrium position of the bridging proton,[22b] this reaction is likely to compete with electron-transfer (ET) reactions.

Use of the PI Yields of DNA Model Compounds To Predict the Hole-Trapping Efficiency in Long-Range DNA Oxidation by Correlating These with Theoretically Calculated Ionization Potentials. On the basis of the preceding discussion on the reported near threshold PI yields for various dinucleotides, it is possible to suggest that these can be directly related to the dinucleotide IPs in aqueous solutions. Furthermore, it was implied in the discussion that the net PI yields and thus the IPs of the dinucleotides are affected by such properties as base sequence, stacking, conformation, and structural fluctuations. In this section, evidence is presented for a direct correlation of the near threshold PI yields (Table 1) with the average IPs values calculated recently for various B-form DNA triads (Table 6 and Figure 4).[11b] The justification for the comparison of the PI yields of the dinucleotides (5′-BX-3′) with the IPs calculated for the triads (5′-XBY-3′) relies on the observation that the neighboring base at the 5′-position in the triads 5′-XBY-3′ does not have an appreciable effect on the IPs of the duplexes.[11b] For instance, for the triad 5′-XGG-3′, the reported IP values are 7.304, 7.305, 7.330, and 7.340 eV for X = G, A, T, and C, respectively.[11b] These values differ by less than 0.04 eV independently of the base at the 5′-end. Thus, it was assumed that the average IP value of the 5′-XGG-3′ triad, namely ⟨X⟩GG = 7.320 eV, can be used to estimate the IP of the dinucleotide GpG (Table 6). With a similar reasoning, the IPs of the other dinucleotides listed in Table 6 were estimated. The fairly good linear correlation between the IP of the triads and the PI yields of the dinucleotides, considering the many approximations made in the calculation of the IP by theoretical methods (see below), further supported their use.[43] The PI trends of the dinucleotides correlate also well with the estimated IPs for double stranded/B-form DNA of the 5′-TXBYT-3′ sequence obtained using ab initio calculations at the HF/6-31G(d) level of theory (not shown),[9c] suggesting that as the distance beyond a few base pairs increases, a smaller effect on the IPs of the 5′-XBY-3′ triads is observed.

The experimental estimation of the IPs (or oxidation potentials) of small oligonucleotides is of fundamental importance for the understanding of charge-transfer efficiency in DNA. Due to the fairly good correlation (Figure 4) between the PI yields and the calculated average IPs, we suggest that the near threshold PI yields of the dinucleotides can be used to estimate the hole-trapping efficiency in DNA and the relative oxidation potentials of DNA model compounds in aqueous solutions. This is based on the fact that if the hole is trapped at the lowest oxidation potential site of the DNA helix, then the hole-trapping efficiency in long-range DNA oxidation should follow the same relative order of the nucleobases' IPs.[9c] The advantage of using near threshold PI yields of DNA model compounds, as proposed, over theoretically calculated gas-phase IPs[9c,11b] lies on the possibility of designing experiments to study such effects as structural fluctuations, conformation, and base stacking at room temperature; sugar−phosphate back-bond structures and their interactions; base pairing; and electrostatic and solvation effects on the charge-transfer efficiency in DNA.

Recently, Saito and co-workers[9b] presented a linear correlation between the relative susceptibility toward one-electron oxidation and the calculated IPs of various triads. They proposed that these IPs could be used to predict the relative reactivity toward one-electron oxidation in DNA. Similar conclusions were reached by Rösch and co-workers.[11b] It is relevant to point out that in these theoretical calculations of the IPs[9c,11b] a static model was used, and the electronic interactions of nucleobases with charged sugar−phosphate fragments and the polar environment (water and counterions) were not taken into consideration. Zhu and LeBreton have examined the effect of the sugar phophate backbone and of counterion by ab initio and ZINDO semiempirical calculations on the PI localization in DNA sequences.[44] They found that the lowest energy base ionization occurs from the interior of the sequence containing multiples repeating guanines (G runs) upon consideration of the sugar and phosphate groups instead of the guanine at the 5′-end reported previously.[45] More recently, it was demonstrated[13] that structural fluctuations in aqueous DNA can induce changes of the order of 0.5 eV in its IPs and earlier UV photoelectron studies demonstrated that a sugar substituent at the pyrimidine N1 and purine N9 causes a reduction of the vertical IP of the free nucleobase of up to 0.6 eV.[46] Indeed, the near threshold PI yields of a wide set of purine and pyrimidine DNA components exposed to 266 nm

laser pulses in aqueous solution[19] and ab initio calculations at the MP2/6-31++G(d,p) level of theory[47] demonstrate a decrease in the IP of the bases with the incorporation of a sugar and/or a sugar phosphate group to the base. Thus, an evident need exists to determine experimentally the relative oxidation potentials of the nucleobases in DNA to be able to predict the efficiency of hole-trapping and the long-range oxidation mechanism in DNA.

In general, higher net PI yields were obtained for the guanine containing dinucleotides (at a similar initial absorbance), in good agreement with the known fact that guanine is the most effective hole trap site in DNA.[9b,48] Inspection of Table 1 suggests that the stabilization of the IP of guanine or adenine is considerably influenced by the subsequent base (except for ApG and GpA), also in very good agreement with previous suggestions.[9c] The most easily oxidized dinucleotide was GpG and the stabilizing effect of 3′-Y guanine neighbors decreased in the order GpG > ApG ≈ TpdG > CpG. In addition, the PI yield was higher in GpC than in CpG, suggesting that the 5′-G terminal is more easily oxidized than the 3′-G. These results are consistent with the observation that the 3′-side G of doublets GG or triplets GGG is far less reactive than the adjacent guanine.[9b,11b] Also, CpA showed the lowest PI yield of the examined dinucleotides and thus the highest IPs (Table 1). This result can probably be explained in terms of a decrease in the electronic coupling at this position, which can increase the tunneling energy gap at this site in DNA, thus reducing long-range charge transport in DNA. This is consistent with the finding that the transmission of charge transfer in DNA is strongly decreased by the insertion of a CA base pair.[5d]

Finally, assuming that the PI yields of the dinucleotides can be related to the oxidation potentials of the bases, our results suggest that the very similar PI yields measured for most of the dinucleotides (Table 1), could imply that the tunneling energy gap of the bases in DNA is small. The estimation of the energy gaps between the bases is fundamental for proposing effective models of charge transport in DNA.[5d] For instance, small energy gaps between the bases could allow for the participation of phonon-assisted hopping in the mechanism of charge transport in long-range DNA oxidation, as suggested by Schuster and co-workers.[5f]

Conclusions

The 266 nm nanosecond laser excitation of a wide range of dinucleotides and related nucleosides and nucleotides leads to their PI through a combination of a one- and two-photon pathways. Sequence, conformation, and base stacking of the dinucleotides were shown to play an important role in the IPs and photoreactivity of these DNA model compounds. These conclusions are based on the observed values of the PI yields at different concentrations, the photodestruction yields, and the competition reactions using electron scavengers. An increase in base stacking was suggested to explain an increase in (1) the net PI yields, (2) the photodestruction, and (3) the participation of the oxidized base in the photodestruction processes. This is probably a consequence of an increase in the electronic coupling and decrease in the tunneling energy gap of the stacked bases as the structural fluctuations of the dinucleotides decrease due to efficient stacking interactions.

The good linear correlation between the PI yields and the calculated IPs of 3- and 5-mer oligonucleotides was used as a convenient and simple way to predict the hole-trapping efficiency in long-range DNA oxidation and to test various properties that have been suggested to regulate the charge transport mechanism in DNA. In this respect, some interesting correlations were presented. The results of this work suggest the possible use of triplets and other small oligonucleotides to better understand the charge-transfer mechanism in short DNA segments and to propose and/or validate the importance of different intrinsic and extrinsic properties characteristic of DNA such as base stacking, base paring, sequence effects, solvation, structural fluctuation, and dynamics on the charge-transfer mechanism and efficiency in B-form DNA.

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(19) Crespo-Hernández, C. E.; Flores, S.; Torres, C.; Negrón-Encarnación, I.; Arce, R. *Photochem. Photobiol.* **2000**, *71*, 534−543. In this previous work the monophotonic PI yield of guanosine was calculated to be 0.083, much higher than that presented in this and in a recent publication (ref 21). The reason for such a drastic discrepancy in the one-photon PI yield was the use of 6-methylpurine (6MP) as a reference actinometer in the former work and the calculation of the PI yield by the comparative method. In that calculation, it was assumed that 6MP photoionizes by a one-photon pathway at 266 nm, but later it was demonstrated by our group that the PI of 6MP occurs through a combination of one- and two-photon pathways and thus the PI yield cannot be calculated at a single laser energy (ref 21). Furthermore, due to the linear increase of the hydrated electron signal as a function of the laser energy, it was assumed that only a one-photon PI was occurring, and not a combination of one- and two-photon, as shown in a later work (ref 21). Thus, the PI yield of guanosine was incorrectly calculated in the former work, resulting in a value differing by almost an order of magnitude.

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