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LASER DIRECT BIPHOTONIC PHOTOLYSIS OF GUANINES WITHIN DNA

Z. SHAQIRI[a,1], A. VESELI[b], N. SHAQIRI[c], E. VIDOLOVA-ANGELOVA[a] and D. ANGELOV[a]

[a]*Institute of Solid State Physics, Bulgarian Academy of Sciences, Tzargradsko Chaussee blvd. 72, BG-1784, Sofia, Bulgaria*

[b]*Faculty of Natural and Mathematical Sciences, 38000, Prishtina, Kosovo*

[c]*Academy of Medicine blvd. 1, G. Sofiski, Sofia, Bulgaria*

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Nanosecond laser-induced oxidative lesions at guanines within DNA were investigated using sequencing gel electrophoresis analysis. Strong sequence-specific lesions at guanines were revealed by either Fpg or piperidine treatments and assigned to 8-oxoG and oxazolone, respectively. It was shown that both the biphotonic ionization process and the chemical reactivity of the radical cation (G^+) are dependent on the DNA sequence. The former were explained by the occurrence of energy and charge migration phenomena, while the latter in terms of local DNA hydration peculiarities.

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1. *Introduction*

One of the fundamental problems of molecular biology and physical chemistry is to understand how local DNA structural deformation or deformability induced by the primary sequence is involved in processes of the DNA-protein interactions. It is now clear that DNA sequence displays a succession of local microheterogeneities which could be described in structural terms such as base twist, roll, tile angles, etc. Local conformational properties of nucleic acids have been shown to play an essential role in biological processes involving DNA-ligand interactions as well as

[1]Corresponding author, e-mail address: zekiinst@yahoo.com

nucleosome packaging and positioning [1]. Recent crystallographic and NMR data have shown a great variability of the structural parameters describing base alignment. These secondary structure peculiarities determine the sites of recognition and binding of various ligands and regulatory proteins. A set of currently available techniques, such as electron microscopy, neutron, photon and X-ray scattering, nuclear magnetic resonance, footprinting by chemically or photonically activated agents, band shift electrophoresis, etc., provide static information to a spatial nucleobases structure.

The flexibility of the photochemical approach has justified its use as a conformational probe of DNA and DNA-protein complexes [2]. The photodamage occurring upon UV lamp irradiation predominantly consists of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoadducts (PyoPyr s). These monophotonic photolesions have been found to be strongly dependent on DNA local conformation in terms of favourable mutual orientation of adjacent reacting bases and deformations induced by interacting proteins. The use of enzymatic chemical and photochemical probes in fingerprinting experiments enables one to obtain a relatively detailed molecular description of a large number of DNA-protein complexes [3–5]. However, none of these techniques possess a sufficient time resolution for the study of the dynamics of the conformational changes occurring in the course of a real biological process such as transcription or replication.

The use of a single short laser pulse for inducing specific DNA lesions might provide an attractive alternative for overcoming the lack of time resolution of existing techniques. The rapid method of photofingerprinting is based on irradiation with high-intensity UV laser pulses [6,7]. It involves a completely different photochemistry, characterized by biphotonic excitation and generation of transient base cation radicals, leading to mono- and pseudo monomolecular products [8-10]. In these investigations, an interesting subject is the occurrence of energy [5] and charge transfer [11–20] in DNA. The redistribution of reactive excited intermediates through energy and charge transfer phenomena are expected to be DNA sequence and structure dependent. It has been already reported that UV laser pulses induce strand breaks [9,19,21] and piperidine labile lesions at guanines [22]. However, the nature of the DNA conformational dependence (photophysical and/or chemical) has not been unambiguously established.

In the present work, by means of the sequencing gel analysis, we show that guanine radical cation is likely to be generated by energy-migration-mediated biphotonic ionization followed by hole migration and fixation by guanines. In addition, the competitive deprotonation versus water addition transformation pathways of the guanine radical cation, leading to the formation of oxazolone and 8-oxoG, respectively, depends on DNA primary sequence and secondary structure.

2. Materials and methods

Olygodeoxynucleotides (for the sequence see Fig. 1) were synthesized in Applied Biosystem (CEA-Grenoble, France) and purified by 15% denaturing PAGE.

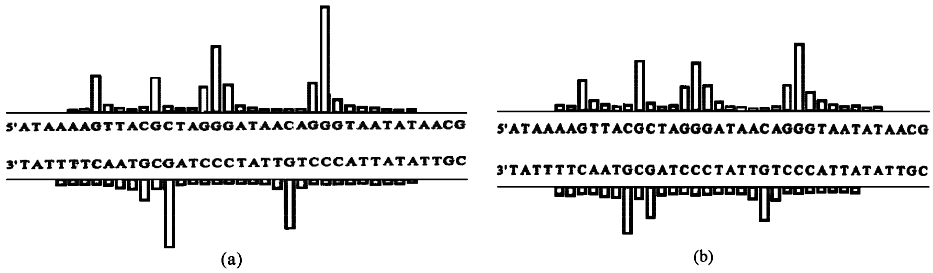

Fig. 1. Distribution of radioactivity in a 15% denaturing page of irradiated ($E = 0.1$ J/cm^2, one pulse) and treated with (a) Fpg and (b) piperidine. The most intensive band accounts for 3.5% of the total radioactivity loaded.

Oligonucleotides were 5' end-labeled with [γ-^{32}P]ATP (Amersham) in the presence of T4 polynucleotide kinase (New England Biolab). DNA duplexes were prepared by annealing equal amount of the labeled and unlabeled complementary strands upon brief heating at 85 °C in TEN buffer: 10 mM Tris, pH8; 1mM EDTA; 30 mM NaCl 10 mM. The fourth harmonic of a Nd:YAG laser ($\lambda = 266$ nm, 15 ns pulse duration) was used for irradiation of 10 μl aliquots in 0.5 ml siliconised Eppendorf tubes as described in Ref. [19].

Irradiated samples were submitted to 1M hot piperidine for 30 minutes at 90 °C or alternatively incubated with 5 ng of Fpg protein for 30 minutes at 37 °C (a gift from S. Boiteux, CEA-Fontenay-aux-Roses, France), lyophilized, resuspended in formamide and run on 15% denaturing polyacrylamide gel. Following electrophoresis, gels were dried, exposed overnight to a phosphorimager screen and quantified by using the ImageQuant v. 4.1 software (Molecular Dynamics). In all experiments the measurement error was less than 5% for the stronger guanine bands. The total cleavage yield never exceeded one break per DNA strand.

3. Results and discussion

The electrophoretic pattern of the irradiated DNA fragments (dose up to $E = 0.1$ J/cm^2 per pulse) without treatment displayed a smear ladder of radioactive background with a poorly expressed base specificity and clear-cut bands (not shown). Contrary to some earlier observations [21], and in accordance with [23], no base-specific strand breaks were observed.

However, after treatment with piperidine or Fpg, strong clear-cut bands appeared, corresponding to cleavage at guanines (see Fig. 1a and 1b) and migrating similarly to Maxam-Gilbert sequence. The distributions of the band intensities under both treatments were strongly sequence dependent. However, the average yield of Fpg-sensitive lesions (FSL) was about two times higher than that of piperidine-sensitive lesions (PSL). Interestingly, the ratio of FSL and PSL cleavage yields was not a constant, but varies from one guanine to another. Upon increasing the laser pulse dose, and keeping the total irradiation dose constant, an increase of the pho-

tolesion yield was observed (see Fig. 2). Importantly, the saturation doses Es were different for different guanines in the system, i.e., the distribution of guanine lesions changed with the variation of the laser pulse dose.

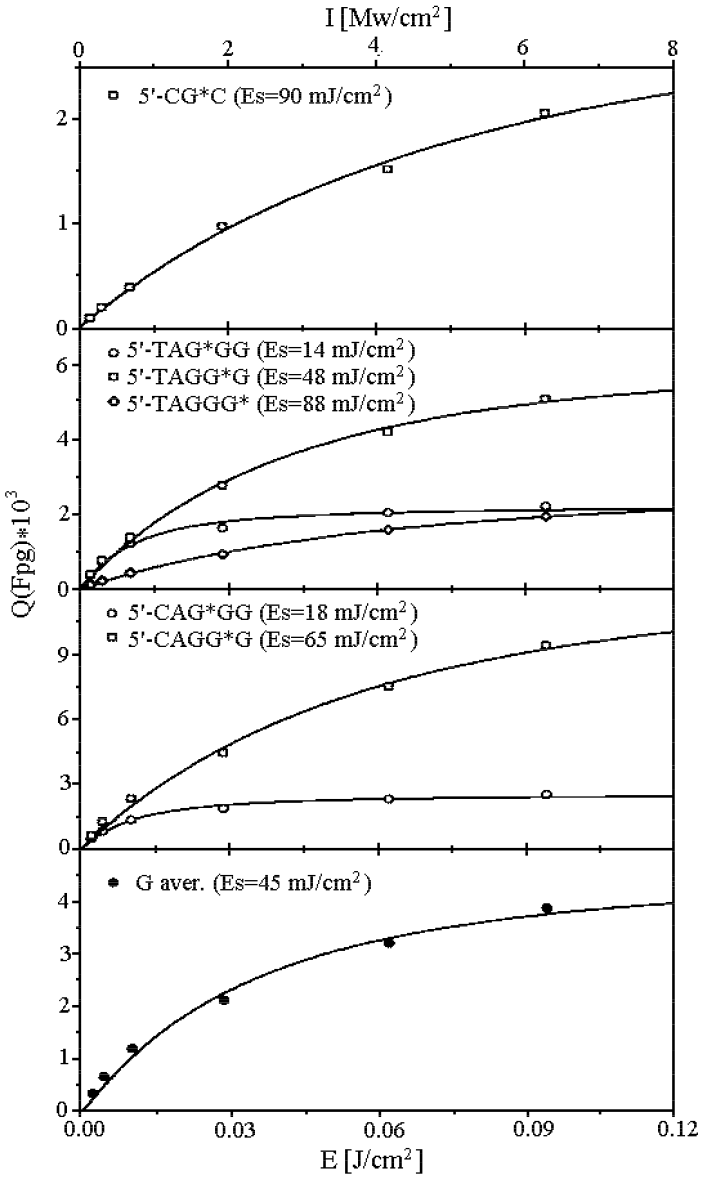

*Fig. 2. Dependence of the quantum yield Q(*Fpg*) on the dose of a single impulse for the guanine bases in the system. E_s is the dose of saturation of Q, corresponding to 0.64 $Q_{\max}$.*

It is well established that the initial reactive species, generated by irradiation of DNA and related model compounds with high-intensity laser pulses are radical cations formed by biphotonic ionization mechanism [8,9] according to the scheme in Fig. 3.

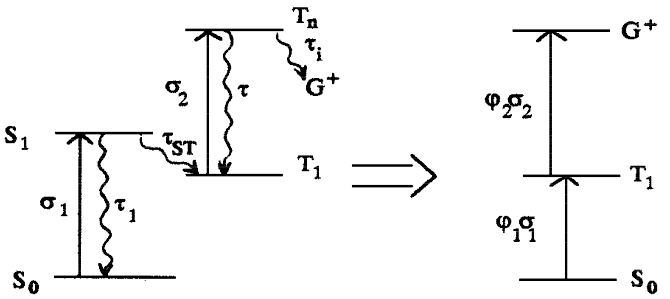

Fig. 3. Simplified scheme of the nanosecond laser biphotonic ionization process of free nucleobases. σ_1 and σ_2 are the absorption cross-sections of the $S_0 \rightarrow S_1$, and $T_1 \rightarrow T_n$ processes, respectively.

In most type 1 photosensitization [11–18] and laser photolysis [19–23] studies, the importance of oxidative damage of guanines was emphasized. It was also established that in DNA, the guanine radical cation decays through deprotonation and hydration resulting mainly in oxazolone and 8-oxoG, respectively [24] (see Fig. 4 showing the chemical structure of oxazolon and 8-8-oxoG). The deprotonation is predominant in isolated nucleosides and to a lesser extent in single-stranded DNA. In double stranded DNA, the hydration was found to prevail, presumably due to partial prevention of deprotonation by stacking interactions [19].

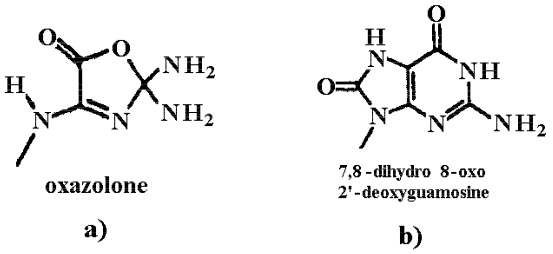

Fig. 4. Chemical structure of a) oxozolon and b) 8-8-oxoG.

The newly formed 8-oxoG, located in the major groove, is recognized and cleaved in an enzyme-catalyzed reaction by the Fpg glycosylase in a sequence-nonspecific process [25]. Having in mind the model of Fpg activity, the deprotonation products of guanine radical cation, especially oxazolone, are not expected to be a good substrate for Fpg. In contrast, this lesion is extremely labile to alkali, while 8-oxoG is poorly affected by piperidine (less than 5%) [23]. Our control experiments showed that the "overlapping" of these two treatments does not exceed 10–15%. Consequently, the comparison of the corresponding electrophoretic patterns reflects

quantitatively the ratio of deprotonation (oxazolone) and hydration (8-oxoG) of the guanine radical cation (Figs. 1a and b).

Our results show that these two reaction pathways are competitive and sequence dependent. Unfortunately, we cannot unambiguously say whether hydration or deprotonation varies within the local conformation. However, taking into account the bimolecular nature of the 8-oxoG formation, we can tentatively anticipate that the water addition plays the determining role in that competition. In other words, the laser photochemistry of DNA could be a unique tool for the study of local DNA hydration [26,27].

As seen from Fig. 2, the generation of the guanine radical cation is sequence dependent. The variation of the quantum efficiency with fluence (Fig. 2) demonstrates that the nature of the guanine photolesions is biphotonic. The experimental points were fitted by the curve calculated using the formula (1) of Refs. [8,19]

$$Q(G^+) = \frac{1}{\sigma_1 E_t}\left(1 - \frac{\mathrm{e}^{-\varphi_1\sigma_1 E}}{1-(\varphi_1\sigma_1)/(\varphi_2\sigma_2)} - \frac{\mathrm{e}^{-\varphi_2\sigma_2 E}}{1-(\varphi_2\sigma_2)/(\varphi_1\sigma_1)}\right). \qquad (1)$$

where $Q(\mathrm{G}^+)$ is the relative quantum efficiency of biphotonic ionization, E_t is the total and E the pulse dose of irradiation, σ_1 and σ_2 are the absorption cross-sections of the ground singlet state S_0 ($\sigma_1 = 2.3 \times 10^{-17}$ cm^2) and excited triplet T_1 states, respectively, φ_1 and φ_1 are the intersystem and ionization conversion yields, respectively.

It should be noted that under the conditions $\varphi_2\sigma_2 \gg \varphi_2\sigma_2$, the maximum value of Q is determined by the values of φ_1, while the saturation intensity E_s is inversely proportional to $\varphi_2\sigma_2$ and almost independent of $\varphi_1\sigma_1$ [8,19]. The comparison of our data for DNA with those for isolated bases [8] shows that E_s is about ten times lower for DNA. In addition, in double-stranded DNA only, E_s depends on the arrangement of guanines, especially in the GGG and GG runs. Having in mind the available literature data, we can conclude that the only physical phenomenon that might account for the great variation of φ_2 for different guanines is the occurrence of electron migration and trapping of the hole by guanines displaying the lowest ionization potential. Following the same logic, the variation of the maximum quantum yield of cleavage (i.e., φ_1) from one guanine to another can be explained by the occurrence of energy migration. The bases, possessing the lowest located triplet state (most probably thymine residues [3]) should be the preferential acceptor targets of the energy within the first excitation step.

4. Conclusions

The chemical transformation pathway of the guanine radical cation in DNA as well as the probability of its generation under two-quantum laser photolysis are sequence dependent. The sequence dependence of the chemical reactivity manifests in the competition between the deprotonation and hydration processes, associated with the local hydration of DNA. Energy and charge migration phenomena within

DNA are likely to mediate the sequence dependence of the photoionization probability. These processes lead to a redistribution of the initially triplet-state excited bases, and further of the radical cation, which is ultimately trapped by the guanine residues.

References

[1] B. Audit, C. Thermes, C. Vaillant, Y. d'Aubenton-Carafa, J. F. Muzi and A. Arneodo, Phys. Rev. Lett. **86** (2001) 2471.

[2] P. E. Nielsen, J. Mol. Recognition **3** (1990) 1.

[3] J. Cadet and P. Vigny, in Bioorganic Photochemistry, ed. H. Morisson, Willey, New York (1990) 1.

[4] M. Becker and J. C. Wang, Nature **309** (1984) 682.

[5] M. Becker and Z. Wang, J. Mol. Biol. **210** (1989) 429.

[6] D. Angelov, M. Berger, J. Cadet, C. Marion and A. Spassky, Trends in Photochem. and Photobiol. **3** (1994) 643.

[7] D. Angelov, E. Novakov, S. Khochbin and S. Dimitrov, Biochem. **38** (1999) 11333.

[8] D. Nikogosyan, D. Angelov and A. Oraevsky, Photochem. Photobiol. **35** (1982) 627.

[9] D. Nikogosyan, Int. J. Radiat. Biol. **57** (1990) 233.

[10] D. Angelov, M. Berger, J. Cadet, N. Getoff and E. Keskinova, S. Solar. Radiat. Phys. Chem. **37** (1991) 717.

[11] S. Kelley and J. K. Barton, Science **283** (1999) 375.

[12] E. Meggers, M. E. Michel-Beyerle and B. Giese, J. Am. Chem. Soc. **120** (1998) 12950.

[13] B. Giese, J. Amaudrut, A.-K. Kohler, M. Spormann and S. Wessely, Nature **412** (2001) 318.

[14] M. Bixon, B. Giese, S. Wessely, T. Langenbacher, M. E. Michel-Beyerle and J. Jortner, Proc. Natl. Acad. Sci. **96** (1999) 11713.

[15] P. T. Henderson, D. Jones, G. Hampikian, Y. Kan and G. B. Schuster, Proc. Natl. Acad. Sci. **96** (1999) 8353.

[16] C. Wan, T. Fiebig, O. Schiemann, J. K. Barton and A. H. Zewail, Proc. Natl. Acad. Sci. **97** (2000) 14052.

[17] K. Nakatani, C. Dohno and I. Saito, J. Am. Chem. Soc. **121** (1999) 10854.

[18] Y. A. Berlin, A. L. Burin and M. A. Ratner, J. Am. Chem. Soc. **123** (2001) 260.

[19] D. Angelov, M. Berger, A. Spassky and J. Cadet, J. Am. Chem. Soc. **119** (1997) 11373.

[20] T. Douki, D. Angelov and J. Cadet, J. Amer. Chem. Soc. **123** (2001) 11360.

[21] D. W. Croke, C. Blau, OhUigin, J. Kelly and D. McConnell, Photochem. Photobiol. **47** (1988) 527.

[22] O. Kovalsky, I. Panyutin and E. Budowsky, Photochem. Photobiol. **52** (1990) 509.

[23] P. M. Cullis, M. E. Malone and L. A. Merson-Davies, J. Am. Chem. Soc. **118** (1996) 2775.

[24] J. Cadet, M. Berger, G. Bucko, L. Ravanat and H. Kasai, in *Frontiers of Photobiology*, Elsevier, Amsterdam (1993) p. 49.

[25] J. Tchou, V. Bodepudi, S. Shibutani, I. Antoshechkin, J. Millers, A. Grollman and F. Johnson. Biol. Chem. **269** (1994) 15318.

[26] W. G. Wamer, W. C. Timmer, R. R. Wei, Sh. A. Miller and A. Kornhanser, Photochem. Photobiol. **61** (1995) 336.

[27] L. A. Lipscomb, M. E. Peek, M. L. Morningstat, S. M. Verghis, E. M. Miller, A. Rich, J. M. Essigmann and L. D. Williams, Proc. Natl. Acad. Sci. USA **92** (1995), 719.

LASERSKA IZRAVNA DVOFOTONSKA FOTOLIZA GUANINA U DNK

Istraživali smo lomove DNK izazvane nanosekundnim laserskim impulsima primjenom gel elektroforeze za određivanje nizova. Otkrili smo snažne nizovno specifične lomove kod guanina djelovanjem sa Fpg ili piperidinom i oni se pripisuju 8-oxoG odnosno oxazolonu. Pokazali smo da su i dvofotonski ionizacijski proces i kemijska reaktivnost radikala kationa (G^+) nizovno ovisni. Prvi se objašnjava selenjem energije i naboja, a drugi kao posebnosti lokalnog hidriranja DNK.