Free Rad. Res., Vol. 21, No. 2, pp. 75-83
Reprints available directly from the publisher
Photocopying permitted by license only

SINGLET OXYGEN INDUCES PREDOMINANTLY G TO T TRANSVERSIONS ON A SINGLE-STRANDED SHUTTLE VECTOR REPLICATED IN MONKEY CELLS

DENISE T. RIBEIRO, REGINA COSTA DE OLIVEIRA[+], PAOLO DI MASCIO[&] and CARLOS F.M. MENCK*

Depto. de Biologia, Instituto de Biociências, Universidade de São Paulo, CP 11461, São Paulo, 05422-970, São Paulo, Brazil

(Received November 23rd, 1993; in revised form February 25th, 1994)

To elucidate the mechanisms of mutagenesis by singlet oxygen DNA damage in mammalian cells, a SV40-derived single-stranded shuttle vector was exposed to the water soluble endoperoxide 3,3'-(1,4-naphthylidene) dipropionate ($NDPO_2$). The damaged vector was transfected into monkey COS7 cells and the plasmid progeny exhibited up to 10 fold increase on the mutation frequency in the *supF* target gene, when compared to untreated vector. The sequence in the *supF* locus of such mutants revealed that singlet oxygen-induced mutagenesis in single-stranded vector is significantly different from spontaneous mutagenesis. Among the base substitutions, most of the mutations involved deoxyguanosines, being G to T transversions the predominant type of change. The data indicate that mutagenesis by singlet oxygen in mammalian cells may be generated by an error prone bypass of damaged deoxyguanosines at the template DNA.

KEY WORDS: Singlet oxygen, mutagenesis, DNA damage, 8-hydroxydeoxyguanine, shuttle vector.

INTRODUCTION

The deleterious effects of the reactive oxygen species are being intensively studied in the last years due to their probable involvement with human ageing, tumor formation and related diseases (1). Among these species, singlet oxygen ($^1O_2$) has been in focus because it has been shown to be generated in several biological systems and it is highly reactive with macromolecules, including nucleic acids and proteins (2,3). Concerning DNA, $^1O_2$ induces several different kinds of lesion, with deleterious biological consequences, such as molecule inactivation and mutagenesis (4,5). The high mutagenicity of $^1O_2$ is a matter of particular interest. Early work have suggested the involvement of $^1O_2$ in the mutagenesis induced by activation of photosensitizers in bacteria (6), yeast (7) and mammalian cells (8). Working with M13 bacteriophage RF DNA treated with photosensitizer, Piette and Van de Vorst (9) and Decuyper-Debergh *et al.* (10) have reported that $^1O_2$ DNA lesions were

*Corresponding author: Dr Carlos F.M. Menck, Depto. de Biologia, IBUSP, CP 11461, São Paulo, 05422-970, São Paulo, Brazil., Tel. ++55.11.818.7587, ++55.11.869.0404 (home), Fax: ++55.11.818.7416
[+]Present address: Unité de Biochimie et Enzymologie, Institut Gustave-Roussy, 94805, Villejuif CEDEX, France: [&]Depto. de Bioquímica, Instituto de Química, USP, São Paulo, SP, Brazil.

9. D. Chiu, B. Lubin and S.B. Shohet (1982) Peroxidative Reactions in Red Cell Biology. In *Free Radicals in Biology* (ed. W.A. Pryor), Academic Press, Orlando, pp. 115–160.
10. J.A. Buege and S.D. Aust (1978) Microsomal lipid peroxidation. *Methods in Enzymology*, **52,** 303–310.
11. M.K. Markwell, S.M. Haas, L.L. Bieber and N.E. Tolbert (1978) A Modification of the Lowry Procedure to Simplify Protein Determination in Membrane and Lipoprotein Samples. *Analytical Biochemistry*, **87,** 206–210.
12. F. Tietze (1969) Enzymic Method for Quantitative Determination of Nanogram Amounts of Total and Oxidized Glutathione: Applications to Mammalian Blood and Other Tissues. *Analytical Biochemistry*, **27,** 502–522.
13. E. Beutler, O. Duron and B.M. Kelly (1963) Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine*, **61,** 882–888.
14. D.J. Hanahan and J.E. Ekholm (1978) The Expression of Optimum ATPase Activities in Human Erythrocytes. *Archives of Biochemistry and Biophysics*, **187,** 170–179.
15. O. Shalev, M.N. Leida, R.P. Hebbel, H.S. Jacob and J.W. Eaton (1981) Abnormal Erythrocyte Calcium Homeostasis in Oxidant-Induced Hemolytic Disease. *Blood*, **58,** 1232–35.
16. H. Fiske and J. Subbarow (1925) The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, **66,** 375.
17. D. Schulte-Frohlinde and C.V. Sonntage (1985) Radiolysis of DNA and Model Systems in the Presence of Oxygen. In *Oxidative Stress* (ed. H. Sies), Academic Press, London and New York, pp. 11–40.
18. R.B. Willson (1985) Organic Peroxy Free Radicals as Ultimate Agents in Oxygen Toxicity. In *Oxidative Stress* (ed. H. Sies), Academic Press, London and New York, pp. 41–72.
19. W.A. Pryor (1986) Oxy-Radicals and Related Species: Their Formation, Lifetimes, and Reactions. *Annual Review of Physiology*, **48,** 657–67.
20. T. Yamada, C. Volkmer and M.B. Grisham (1990) Antioxidant properties of 5-ASA: Potential mechanism for its anti-inflammatory activity. *Canadian Journal of Gastroenterology*, **4,** 1–8.
21. I. Ahnfelt-Ronne, O.H. Nielsen, A. Christensen, E. Landholz, V. Binder and P. Riis (1990) Clinical Evidence Supporting the Radical Scavenger Mechanism of 5-Aminosalicylic Acid. *Gastroenterology*, **98,** 1162–1169.
22. M. Miki, H. Tamai, M. Mino, Y. Yamamoto and E. Niki (1987) Free- Radical Chain Oxidation of Rat Red Blood Cells by Molecular Oxygen and Its Inhibition by α-Tocopherol. *Archives of Biochemistry and Biophysics*, **258,** 373–380.
23. Y. Takenaka, M. Miki, H. Yasuda and M. Mino (1991) The effect of α-Tocopherol as an Antioxidant on the Oxidation of Membrane Protein Thiols Induced by Free Radicals Generated in Different sites. *Archives of Biochemistry and Biophysics*, **285,** 344–350.
24. Y. Yamamoto, E. Niki, J. Eguchi, Y. Kamiya and H. Shimasaki (1985) Oxidation of bilogical membranes and its inhibition. Free radical chain oxidation of erythrocyte ghost membranes by oxygen. *Biochemica et Biophysica Acta*, **819,** 29–26.
25. Y. Yamamoto, E. Niki, Y. Kamiya, M. Miki, H. Tamai and M. Mino (1986) Free Radical Chain Oxidation and Hemolysis of Erythrocytes by Molecular Oxygen and Their Inhibition by Vitamin E. *Journal of Nutritional Science and Vitaminology*, **32,** 475–479.
26. E. Niki, Y. Yamamoto, M. Takahashi, K. Yamamoto, Y. Yamamoto, E. Komuro, M. Miki, H. Yasuda and M. Mino (1988) Free Radical-Mediated Damage of Blood and Its Inhibition by Anti-oxidants. *Journal of Nutritional Science and Vitaminology*, **34,** 507–512.
27. E. Niki, Y. Yamamoto, E. Komuro and K. Sato (1991) Membrane damage due to lipid oxidation. *American Journal of Clinical Nutrition*, **53,** 201S–5S.
28. J.W. Eaton, P.E. Hallaway and N.S. Agar (1989) Erythrocyte Glutathione: A Dispensable Oxidant Defense? *The Red Cell*, The Seventh Ann Arbor Conference. pp. 23–38.
29. J. Reglinski, S. Hoey, W.E. Smith and R.D. Sturrock (1988) Cellular Response to Oxidative Stress at Sulfhydryl Group Receptor Sites on the Erythrocyte Membrane. *Journal of Biological Chemistry*, **263,** 12360–66.
30. S.L. Schrier (1977) Human Erythrocyte Membrane Enzymes: Current Status and Clincial Correlates. *Blood*, **50,** 227–237.
31. F.A.X. Schanne, A.B. Kane, E.E. Young and J.L. Farber (1979) Calcium Dependence of Toxic Cell Death: A Final Common Pathway. *Science*, **206,** 700–702.
32. U. Raess (1989) Pharmacological Modification of the Red Cell Ca^{2+}-Pump. In *The Red Cell membrane: a model of solute transport* (ed. N.J. Clifton), Humana Press, pp. 305–327.

Accepted by Prof. B. Halliwell

carbon (NDP) and molecular oxygen, half in the triplet state and half in the excited

singlet state (12). After treatment, the DNA samples were sterilized and purified with chloroform.

Transfection into COS7 Cells and Shuttling into E. coli

Subconfluent COS7 cells were transfected with treated plasmid DNA (1 μg per 90 mm dish) by the DEAE-dextran technique (21). Three days later the replicated episomal DNA was recovered by a small-scale alkaline lysis procedure adapted from the method described by Birnboim and Doly (22). Then it was shuttled into *E. coli* MBM7070 by the transformation method of Hanahan (23).

Mutagenesis Analysis

Transformant colonies were plated on LB-agar medium in the presence of chloramphenicol (34 μg/ml). X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside, 0.08 mg/ml) and IPTG (isopropyl-β-D-thiogalactoside, 0.1 mM). Colonies bearing plasmids with functional *supF* gene are dark blue, while colonies bearing plasmids with inactivating mutations in this gene are white or light blue. These colonies were restreaked three times on the same medium to confirm the phenotype and the plasmid DNA was extracted by a modified alkaline lysis "mini-prep" method (22). The *supF* locus of these plasmids was then sequenced by the Sanger chain termination method (24) using the Sequenase Version 2.0 kit (United States Biochemical Corporation) and a 17mer oligonucleotide, which hybridizes in a position 5′ to the *supF* gene, was used as primer for the sequencing reactions. Only independent mutations are presented: mutants carrying the same modification(s) were considered independent if they were isolated from different transfection experiments in COS7 cells.

RESULTS

Previous work showed that $NDPO_2$-treated single-stranded DNA, πSVPC13FIA vector, yields *supF* mutants after replication in mammalian cells (14). The data presented in Figure 1 show that there is a linear increase up to a factor of 10 in the mutation frequency induced by different $NDPO_2$ concentrations, in comparison to control DNA, that is, single-stranded DNA submitted to the same experimental conditions without $NDPO_2$. As reported before (14,25), the number of lesions induced on single-stranded DNA by $NDPO_2$ was lower when the reaction was performed in the presence of H_2O, instead of D_2O (which prolongs $^1O_2$ lifetime), or NaN_3 (an $^1O_2$ quencher). No lesions were detected when DNA was treated with NDP, a product of the $NDPO_2$ thermolysis. Therefore, the damaging action of $NDPO_2$ is probably due to $^1O_2$. This implies $^1O_2$ must also be the agent responsible for the mutagenic effects described here.

In order to characterize the molecular nature of these mutations, the *supF* locus of the mutant plasmids from control and $^1O_2$-treated DNA were sequenced. The results obtained for independent mutants are grouped in Table 1. The mutations induced by $NDPO_2$-treatment are mainly single and multiple base substitutions, while, for control DNA, mutations are distributed between gross alterations (deletions) and single base substitutions.

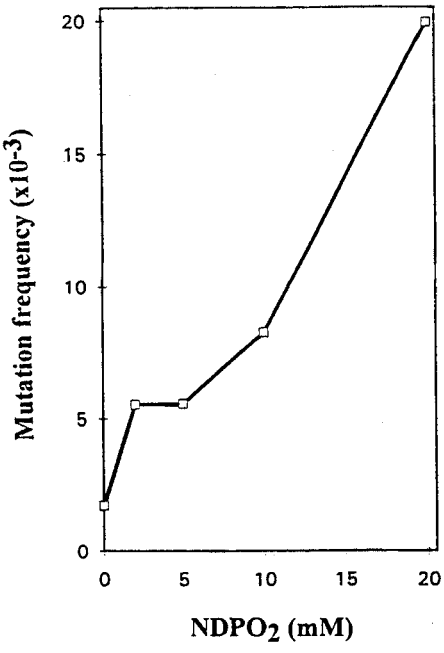

FIGURE 1 Increase in the mutation frequency at the *supF* locus in $^1O_2$-treated single-stranded shuttle vector after replication in monkey COS7 cells. The data are from Ribeiro *et al.* (14).

TABLE 1
Mutations in control and $NDPO_2$-treated single stranded πSVPC13FIA vector replicated in COS7 cells

Types of mutations	Number of mutants	
	Control	$NDPO_2$-treated
deletions	7 (38.9%)[a]	1 (8.3%)[a]
frameshift	1 (5.6%)[b]	0
base substitutions		
single	8 (44.4%)	6 (50.0%)
multiple	2 (11.1%)[c]	5 (41.7%)[d]
total	18 (100%)	12 (100%)

[a] Deletions involve from 17 to 183 b, which include the gene itself and the 5′ region.
[b] (−1) deletion.
[c] One of them involves 2 adjacent bases (tandem) and, the other, 2 bases separated by an unchanged nucleotide.
[d] Three of them include mutations in tandem.

The types of base substitutions are summarized on Table 2. Among the twenty changes found in mutants originated from $^1O_2$-treated DNA, a significant majority involves dGs: 16 modifications, including 12 G to T substitutions. The latter change is the most frequent type of mutation induced by $NDPO_2$ treatment. It should be emphasized that the mutations found in untreated DNA were significantly different from those described above. The main difference is the fact that no transversions involving dGs were detected for control DNA.

TABLE 2
Base substitutions in mutants derived from control and $NDPO_2$-treated single stranded πSVPC13FIA replicated in COS7 cells

	Number of substitutions	
	Control	$NDPO_2$-treated
Transitions	6 (50%)	1 (5%)
C → T	4 (33.4%)	1 (5%)
G → A	2 (16.6%)	0
Transversions	6 (50%)	19 (95%)[a]
C → A	1 (8.3%)	1 (5%)
C → G	4 (33.4%)	2 (10%)
G → C	0	4 (20%)
G → T	0	12 (60%)
A → T	1 (8.3%)	0
Total	12 (100%)	20 (100%)

[a] Spontaneous and $NDPO_2$-treated DNA yields different transversion frequencies statistically significant ($p < 0.01$, Fisher exact test).

DISCUSSION

Mutants in the *supF* locus of single-stranded DNA, originated either spontaneously or after $^1O_2$-treatment and replication in monkey COS7 cells, were characterized by DNA sequencing. The *supF* locus is particularly interesting as mutation target gene since it has been extensively used in shuttle vectors and base changes at almost every site of the structural tRNA coding sequence inactivate the suppressor function (26). Although only few independent mutants were found, due to the low recovery of plasmid in the treated samples (14), the data presented here consistently show significant differences between the mutations originated from control and $^1O_2$-damaged vectors. These differences are observed in three levels: (i) types of mutation; (ii) types of base substitutions and (iii) pattern of distribution of mutations along the *supF* locus (not shown). The data concerning spontaneous mutations are in agreement to those reported before for another SV40-derived single-stranded shuttle vector (27): only one transversion out of 30 independent mutations analysed, involving guanine residue, was found. These types of base substitution are the most frequent ones (80%) observed for $^1O_2$-damaged single-stranded vectors (Table 2).

This work confirms and extends the results obtained for a double-stranded shuttle vector treated with $NDPO_2$ and replicated in mammalian cells (15). In that case, the most frequent base substitution affects G:C base pairs (98%) including G:C to T:A (51%) and G:C to C:G (33%) transversions. In this paper, we observed that 16 of 20 point mutations derived from $^1O_2$-damaged single-stranded vector are targeted to dGs. The use of single-stranded DNA in these experiments eliminates the ambiguity of which strand has been damaged, so that a direct correlation between the types of mutation observed and the site of base damaged can be established (17,28). Moreover, single-stranded DNA is a common structure within the cell (eg. as an intermediate during DNA replication and recombination). Also, the damages in a single-stranded vector cannot be easily repaired. Thus, the data presented here may reflect the consequences of translesion DNA synthesis in the cell nuclei. The mutations clearly occur predominantly opposite to dG templates.

The high frequency of G to T mutations observed in mammalian cells gives clues on the molecular nature of the kind of guanine modifications induced by $^1O_2$. Several lines of evidence indicate that 8-hydroxy-2′-deoxyguanosine (8-oxodG) is an important product of the oxidation of dG in DNA by $^1O_2$ (28–31). This base alteration was found to occur about 17-fold more frequently than single strand breaks (29) and about 20-fold more than an imidazole ring-opened purine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (30) in DNA treated with methylene blue plus light. Devasagayam *et al.* (32) have observed that exposure of free nucleotides to $NDPO_2$ yields 8-oxodG as the main product of dG oxidation by $^1O_2$. Moreover, *in vitro* experiments indicate that 8-oxodG may miscode for deoxyadenosine during DNA synthesis by several DNA polymerases (33) and NMR structural analysis of a oligonucleotide duplex provide evidence that 8-oxodG stably base pairs deoxyadenosine (34). Three independent studies using vectors containing site-specifically inserted single 8-oxodG residues have shown that G to T transversions are the most frequent mutations arising after *E. coli* transformation (35–37). More recently, Moriya (38) reported that a single 8-oxodG in a single-stranded shuttle vector is mutagenic when replicated in COS7 cells, resulting in G to T transversions. Therefore, 8-oxodG is a strong candidate as the major premutagenic lesion induced by $^1O_2$, yielding G to T transversions in mammalian cells.

Recently, McBride *et al.* (11) have found that, for single-stranded DNA exposed to methylene blue plus light, mutagenesis in *E. coli* is highly dependent on SOS response. The mutations predominantly observed were also base substitutions occurring at template G residues. However, G to C transversions were the most frequent mutations (about 58%), with G to T transversions as the second highest frequent mutations. The G to C transversions were also identified in the system described here and for double-stranded shuttle vectors treated with $^1O_2$ (15). This and the other types of mutation observed may be attributed to minor lesions present in $^1O_2$-damaged DNA, such as phosphodiester chain breaks (39,40), alkali and piperidine-labile sites (39) and formamidopyrimidine (30).

The data presented here also reveal that part of the mutants contains multiple base substitutions. This was also verified for double-stranded shuttle vectors with lesions induced by $^1O_2$ (15) or other damaging agents (41–44). Seidman *et al.* (43) proposed that multiple mutations arise as result of an error DNA polymerization during excision repair. Excision repair is however unable to act on single-stranded DNA due to the absence of template. Moreover, multiple mutations were also reported for UV-irradiated single-stranded shuttle vectors (27,45). As in this work, these authors have also found that UV mutagenesis is targeted to damaged bases, pyrimidine doublets in that case. Therefore, it seems that at least part of the multiple mutations observed in shuttle vectors may be due to the presence of two or more close lesions on the target gene.

In conclusion, our work using a $^1O_2$-damaged single stranded vector indicates that $^1O_2$-mutagenesis in mammalian cells occur by the miscoding at template modified G residues during DNA replication. The major premutagenic lesion may be 8-oxodG, which mainly generates G to T transversions. The biological importance of mutagenic events presented here is directly related to the occurrence of $^1O_2$ within the cell nuclei.

Acknowledgements

We thank Dr. A. Sarasin and Dr. H. Sies for their incentive since the beginning of this work. We are also grateful to Dr. Sergio Matioli for his collaboration on the preparation of this manuscript. This project was supported by the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, São Paulo, Brazil) and Conselho Nacional para o Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Brazil). R.C.O. and P.D.M. are recipients of postdoctoral fellowships from CNPq.

References

1. P. Cerutti (1985) Prooxidant states and tumor promotion. *Science*, **227**, 375–381.
2. A. Naqui, B. Chance and E. Cadenas (1986) Reactive oxygen intermediates in biochemistry. *Annual Review of Biochemistry*, **55**, 137–166.
3. B. Halliwell and J.M.C. Gutteridge (1989) *Free Radical in Biology and Medicine*. 2nd ed., Clarendon Press, Oxford.
4. H. Sies and C.F.M. Menck (1992) Singlet oxygen induced DNA damage. *Mutation Research*, **275**, 367–375.
5. H. Sies (1993) Damage to plasmid DNA by $^1O_2$ and its protection. *Mutation Research*, **299**, 183–191.
6. B. Gutter, W.T. Speck and H.S. Rosenkranz (1977) A study of the photoinduced mutagenicity of methylene blue. *Mutation Research*, **44**, 177–182.
7. K. Kobayashi and T. Ito (1976) Further *in vivo* studies on the participation of singlet oxygen in the photodynamic inactivation and induction of genetic changes in *Saccharomyces cerevisiae*. *Photochemistry and Photobiology*, **23**, 21–28.
8. N. Gruener and M.P. Lockwood (1979) Photodynamic mutagenicity in mammalian cells. *Biochemical and Biophysical Research Communications*, **90**, 460–465.
9. J. Piette and A. Van de Vorst (1984) Targeted mutagenesis of M13 DNA induced by photosensitized reaction. *Photochemistry and Photobiology*, **40**, 635–640.
10. D. Decuyper-Debergh, J. Piette and A. Van de Vorst (1987) Singlet oxygen induced mutations in the M13lacZ phage DNA. The *EMBO Journal*, **6**, 3155–3161.
11. T.J. McBride, J.E. Schneider, R.A. Floyd and L.A. Loeb (1992) Mutations induced by methylene blue plus light in single-stranded M13mp2. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 6866–6870.
12. P. Di Mascio and H. Sies (1989) Detection and quantification of singlet oxygen generated by thermolysis of a water-soluble endoperoxide. *Journal of the American Chemical Society*, **111**, 2909–2914.
13. P. Di Mascio, C.F.M. Menck, R.G. Nigro, A. Sarasin and H. Sies (1990) Singlet oxygen induced mutagenicity in a mammalian SV40-based shuttle vector. *Photochemistry and Photobiology*, **51**, 293–298.
14. D.T. Ribeiro, C. Madzak, A. Sarasin, P. Di Mascio, H. Sies and C.F.M. Menck (1992) Singlet oxygen induced DNA damage and mutagenicity in a single-stranded SV40-based shuttle vector. *Photochemistry and Photobiology*, **55**, 39–45.
15. R. Costa de Oliveira, D.T. Ribeiro, R.G. Nigro, P. Di Mascio and C.F.M. Menck (1992) Singlet oxygen induced mutation spectrum in mammalian cells. *Nucleic Acids Research*, **20**, 4319–4323.
16. C. Madzak, C.F.M. Menck, J. Armier and A. Sarasin (1989) Analysis of single-stranded DNA stability and damage-induced strand loss in mammalian cells using SV40-based shuttle vectors. *Journal of Molecular Biology*, **205**, 501–509.
17. J.B. Cabral-Neto, A. Gentil, R.E.C. Cabral and A. Sarasin (1992) Mutation spectrum of heat induced abasic sites on a single stranded shuttle vector replication in mammalian cells. *The Journal of Biological Chemistry*, **267**, 19718–19723.
18. Y. Gluzman (1981) SV40-transformed simian cells support the replication of early SV40 mutants. *Cell*, **23**, 175–182.
19. C. Yanish-Perron, J. Vieira and J. Messing (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene*, **33**, 103–119.
20. J. Sambrook, E.F. Fritsch, and T. Maniatis (1989) *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
21. J.H. Wilson (1978) Interference in SV40 DNA infection: a possible basis for cellular competence. *Virology*, **91**, 380–388.
22. H.C. Birnboim and J. Doly (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research*, **7**, 1513–1523.

23. D. Hanahan (1983) Studies on transformation of *E. coli* with plasmids. *Journal of Molecular Biology*, **166**, 557–580.
24. F. Sanger, S. Nicklen and A.R. Coulson (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, **74**, 5463–5467.
25. D.T. Ribeiro, F. Bourre, A. Sarasin, P. Di Mascio and C.F.M. Menck (1992) DNA synthesis blocking lesions induced by singlet oxygen are targeted to deoxyguanosines. *Nucleic Acids Research*, **20**, 2465–2469.
26. K.H. Kramer and M.M. Seidman (1989) Use of *supF*, an *Escherichia coli* tyrosine suppressor tRNA gene, as a mutagenic target in shuttle vector plasmid. *Mutation Research*, **220**, 61–72.
27. C. Madzak, J.B. Cabral-Neto, C.F.M. Menck and A. Sarasin (1992) Spontaneous and ultraviolet-induced mutations on a single stranded shuttle vector transfected into monkey cells. *Mutation Research*, **274**, 135–145.
28. R.A. Floyd, M.S. West, K.L. Eneff and J.E. Schneider (1989) Methylene blue plus light mediates 8-hydroxyguanine formation in DNA. *Archives of Biochemistry and Biophysics*, **273**, 106–111.
29. J.E. Schneider, S. Price, L. Maidt, J.M.C. Gutteridge and R.A. Floyd (1990) Methylene blue plus light mediates 8-hydroxy-2′-deoxyguanosine formation in DNA preferentially over strand breakage. *Nucleic Acids Research*, **18**, 631–635.
30. S. Boiteux, E. Gajewski, J. Laval and M. Dizdaroglu (1992) Substrate specificity of the *Escherichia coli* FPG protein (Formamidopyrimidine-DNA glycosylase): Excision of purine lesions in DNA produced by ionizing radiation or photosensitization. *Biochemistry*, **31**, 106–110.
31. J. Piette (1991) Biological consequences associated with DNA oxidation mediated by singlet oxygen. *Journal of Photochemistry and Photobiology B: Biology*, **11**, 241–260.
32. T.P.A. Devasagayam S. Steenken, W.A. Schulz and H. Sies (1991) Formation of 8-hydroxy-(deoxy)guanosine and generation of strand breaks at guanine residues in DNA by singlet oxygen. *Biochemistry*, **30**, 6283–6289.
33. S. Shibutani, M. Takeshita and A.P. Grollman (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*, **349**, 431–434.
34. M. Kouchakdjian, V. Bodepudi, S. Shibutani, M. Eisenberg, F. Johnson, A. Grollman and D.J. Patel (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-oxo-7H-dG(*syn*).dA(*anti*) alignment at lesion site. *Biochemistry*, **30**, 1403–1412.
35. M.L. Wood, M. Dizdaroglu, E. Gajewski and J.M. Essigmann (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry*, **29**, 7024–7032.
36. M. Moriya, C. Ou, V. Bodepudi, F. Johnson, M. Takeshita and A.P. Grollman (1991) Site specific mutagenesis using a duplex vector: a study of translesion synthesis past 8-hydroxyguanosine in *E. coli*. *Mutation Research*, **254**, 281–288.
37. K.C. Cheng, D.S. Cahill, H. Kasai, S. Nishimura and L.A. Loeb (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G → T and A → T substitutions. *Journal of Biological Chemistry*, **1**, 166–172.
38. M. Moriya (1993) Single-stranded shuttle phagemid for mutagenesis studies in mammalian studies in mammalian cells: 8-oxoguanine in DNA induces targeted G:C→T:A transversions in simian kidney cells. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 1122–1126.
39. E.R. Blazek, J.G. Peak and M.J. Peak (1989) Singlet oxygen induces frank strand breaks as well as alkali- and piperidine-labile sites in supercoiled plasmid DNA. *Photochemistry and Photobiology*, **49**, 607–613.
40. P. Di Mascio, H. Wefers, H.-P. Do-Thi, M.Y.M. Lafleur and H. Sies (1989) Singlet molecular oxygen causes loss of biological activity in plasmid and bacteriophage DNA and induces single-strand breaks. *Biochimica et Biophysica Acta*, **1007**, 151–157.
41. J. Hauser, M.M. Seidman, K. Sidur and K. Dixon (1986) Sequence specificity of point mutations induced during passage of a UV-irradiated shuttle vector plasmid in monkey cells. *Molecular and Cellular Biology*, **9**, 277–285.
42. S.M. Keyse, F. Amaudruz and R.M. Tyrrell (1988) Determination of the spectrum of mutations induced by defined-wavelength solar UVB (313 nm) radiation in mammalian cells by use of a shuttle vector. *Molecular and Cellular Biology*, **8**, 5425–5431.
43. M.M. Seidman, A. Bredberg, A. Seetharan and K.H. Kraemer (1987) Multiple point mutations in a shuttle vector propagated in human cells: evidence for error-prone DNA polymerase activity. *Proceedings of the National Academy of Sciences of the United States of America*, **84**, 4944–4948.

44. H.C. Hsia, J.S. Lebkowski, P.-M. Leong, M.P. Calos and J.H. Miller (1989) Comparison of ultraviolet-induced mutagenesis of the *lacI* gene in *Escherichia coli* and in human 293 cells. *Journal of Molecular Biology*, **205**, 103–113.
45. C. Madzak and A. Sarasin (1991) Mutation spectrum following transfection of ultraviolet-irradiated single-stranded or double-stranded shuttle vector DNA into monkey cells. *Journal of Molecular Biology*, **218**, 667–673.

Accepted by Professor H. Sies