Induction of Chromosomal Gene Mutations in *Escherichia coli* by Direct Incorporation of Oxidatively Damaged Nucleotides

NEW EVALUATION METHOD FOR MUTAGENESIS BY DAMAGED DNA PRECURSORS *IN VIVO*®

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We have developed a new strategy for the evaluation of the mutagenicity of a damaged DNA precursor (deoxyribonucleoside 5′-triphosphate) in *Escherichia coli*. 8-Hydroxydeoxyguanosine triphosphate (8-OH-dGTP) and 2-hydroxydeoxyadenosine triphosphate (2-OH-dATP) were chosen for this study because they appear to be formed abundantly by reactive oxygen species in cells. We introduced the oxidatively damaged nucleotides into competent *E. coli* and selected mutants of the chromosomal *lacI* gene. Both damaged nucleotides induced *lacI* gene mutations in a dose-dependent manner, whereas unmodified dATP and dGTP did not appear to elicit the mutations. The addition of 50 nmol of 8-OH-dGTP and 2-OH-dATP into an *E. coli* suspension induced 12- and 9-fold more substitution mutations than the spontaneous event, respectively. The 8-OH-dGTP induced A→T and G→C transversions, and the 2-OH-dATP elicited G→C and T→A transversions. These results indicate that the two oxidatively damaged nucleotides are mutagenic in *vivo* and suggest that 8-OH-dGTP and 2-OH-dATP were incorporated opposite A and G residues, respectively, in the *E. coli* DNA. This new method enables the evaluation and comparison of the mutagenic potentials of damaged DNA precursors *in vivo*.

Numerous environmental mutagens and carcinogens are produced endogenously by normal oxygen metabolism and are also produced by environmental mutagens such as ROS, ultraviolet, X-, and γ-rays, and alkylating agents, in addition to mispair formation during replication. Among them, ROS are believed to be very important sources of mutations and to be involved in mutagenesis, carcinogenesis, and aging (1, 2) because ROS are generated endogenously by normal oxygen metabolism and are also produced by many environmental mutagens and carcinogens.

Mutations, alterations of genetic information, are believed to cause various diseases and are generated by many environmental mutagens such as ROS, ultraviolet, X-, and γ-rays, and alkylating agents, in addition to mispair formation during replication. Among them, ROS are believed to be very important sources of mutations and to be involved in mutagenesis, carcinogenesis, and aging (1, 2) because ROS are generated endogenously by normal oxygen metabolism and are also produced by many environmental mutagens and carcinogens.

Among the forms of oxidative DNA damage reported, 8-OH-Gua (3) is recognized as an important lesion because of its mutagenicity (4–9). This modified base is used widely as a marker of DNA oxidation (3, 10) because of its sensitive detection by an HPLC system connected to an electrochemical detector. An oxidized form of adenine, 2-OH-Ade, is produced by Fenton-type reactions of deoxyadenosine derivatives (11, 12). The yields of 2-OH-Ade are similar to those of 8-OH-Gua in the monomeric form, although its formation in DNA is less efficient. It was reported that the treatment of cultured human cells with H₂O₂ induces 2-OH-Ade accumulation in DNA (one-fifth of that of 8-OH-Gua) (13). Moreover, 2-OH-Ade possesses mutation indibility similar to that of 8-OH-Gua in *Escherichia coli* and mammalian cells (14, 15). Thus, 2-OH-Ade appears to be another important form of DNA damage produced by ROS.

An oxidative DNA lesion is likely to be formed through two pathways. One is the direct oxidation of a residue in DNA, and another is the incorporation of an oxidatively damaged DNA precursor by a DNA polymerase(s). In fact, it was shown that the two pathways contribute almost equally to the formation of 8-OH-Gua in DNA (16). Moreover, the presence of the MutT protein and its mammalian counterpart, which hydrolyze the mutagenic nucleotide 8-OH-dGTP, indicates that the prevention of its incorporation into DNA is important in organisms (17, 18). Thus, it is necessary to determine the mutagenicity of an oxidatively damaged DNA precursor in cells to reveal the overall effects on the mutations induced by ROS.

However, few studies on the mutagenic potential of an oxidatively damaged DNA precursor have been reported. 8-OH-dGTP is used as a substrate by DNA polymerases and is inserted opposite C and A in the template DNA (5, 17). We reported the insertion of 2-OH-dAMP opposite T and C by the mammalian DNA polymerase α (11). The incorporation of 5-hydroxydeoxycytidine 5′-triphosphate and 5-formyldeoxouridine 5′-triphosphate opposite A and G has also been reported (19, 20). Minnick et al. (21) used 8-OH-dGTP as a substrate in SV40 origin-dependent DNA replication with the use of a human cell extract (21). All of these studies were conducted *in vitro*, and to our knowledge, the mutagenicity of a damaged nucleotide *in vivo* has never been reported.

In this study, we identified two important oxidatively damaged purine nucleotides, 8-OH-dGTP and 2-OH-dATP (Fig. 1), into *E. coli* and analyzed the induction of mutations in the *lacI* gene on the chromosomal DNA. 8-OH-dGTP and 2-OH-dATP were similarly mutagenic in *vivo* and elicited A→T and G→C→T→A transversions, respectively. This new method enables the evaluation and comparison of the mutagenic potentials of damaged DNA precursors *in vivo*. This paper is available on line at http://www.jbc.org
EXPERIMENTAL PROCEDURES

Materials—All chemicals for the E. coli media were as described (22–24). The E. coli strains W3110 (wild type, F') and DH5α (F', ΔlacZYA-argF) U169, endA1, recA1, hsdS21, (rK- mK+), supE44, λ-, gyrA96, relA1) were used. Recombinant Taq DNA polymerase was purchased from Nacalai Tesque Inc. The dATP used for the preparation of 2-OH-dATP was from Sigma. Nucleotides for control treatments were from Amersham Pharmacia Biotech. Digoxigenin-labeled oligonucleotides and other unmodified oligonucleotides were from Nissinbo (Tokyo, Japan) and from Hokkaido System Science Co. (Sapporo, Japan), respectively, in purified forms.

Preparation of Damaged Nucleotides—8-OH-dGTP was prepared as described previously (5). 2-OH-dATP was prepared by the treatment of dATP with Fe(II)-EDTA-O2 and was purified by HPLC as described (11).

Introduction of Nucleotides into E. coli W3110 Cells and Selection of lacI Mutants—Selection of lacI mutants was carried out by the method of Miller (25). A white colony (the lacI genotype) of W3110 was taken from a 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside mini plate and was inoculated into Luria-Bertani (LB) medium. The E. coli culture was incubated at 37 °C for 2 h, and competent cells were prepared as described (26). To 100 μl of the E. coli suspension, 5 μl of nucleotide solution was added, and the mixture was placed on ice for 30 min. After heat shock treatment (42 °C for 2 min and then 0 °C for 2 min), 800 μl of LB medium was added, and the cells were incubated at 37 °C for 45 min. A portion of the culture was diluted, transferred onto an LB agar plate, and incubated at 37 °C overnight. Another portion of the culture was transferred onto a P-gal plate and was incubated at 37 °C for 3 days. The colonies that grew on the P-gal plate contain a mutation in either lacI or lacO gene and were scored as mutants (25). The mutation frequency (MF) was calculated according to the numbers of colonies on the P-gal and LB plates. The lacI mutants were selected from the mixture of lacI− and lacO− mutants on the P-gal plates as described (25). The isolated mutants were inoculated into 0.5 ml of LB medium and were incubated at 37 °C for 3 days. The chromosomal DNA was isolated from the E. coli cells containing the mutated lacI− gene with a SepaGene kit (Sanko Junyaku). The DNA fragment containing the lacI− gene was amplified by polymerase chain reaction as described previously (22, 23).

Analysis of Mutations—The chromosomal DNA was isolated from the E. coli cells containing the mutated lacI− gene with a SepaGene kit (Sanko Junyaku). The DNA fragment containing the lacI− gene was amplified by polymerase chain reaction as described previously (22, 23).

The presence of an addition or deletion of the 5′-TGCC-3′ sequence, which is detected frequently in the case of spontaneous mutations in the lacI gene, was judged by dot-blot hybridization. The amplified lacI fragments were heat denatured and blotted onto a nitrocellulose membrane (Protran, Schleicher & Schuell) and were fixed by UV-cross-linking. A probe labeled with digoxigenin at the 5′-end was used in the hybridization. The sequences of the probes were 5′-dTCUGCTGCTGC-TGCTGCGCAT-3′ and 5′-dTCUGCTGCTGC-TGCTGCGCATATAA-3′. Hybridization was carried out at 65 °C. Positive signals were detected by the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

The nucleotide sequences of the lacI gene fragments were analyzed by sequencing the polymerase chain reaction products using an Applied Biosystems PRISM Dye Primer Cycle Sequencing Kit (Perkin-Elmer) and an Applied Biosystems model 373S DNA sequencer (Perkin-Elmer) as described previously (22).

Measurement of 8-OH-Gua Content in Chromosomal DNA in E. coli—The E. coli W3110 treated with 8-OH-dGTP as described above was incubated at 37 °C for an additional 60 min in LB medium. After centrifugation, the E. coli pellet was washed with ice-cold LB medium to remove unincorporated 8-OH-dGTP. Control E. coli was treated similarly. The chromosomal DNAs were extracted from 8-OH-dGTP-treated and control bacteria using a DNA Extractor WB Kit (Wako Pure Chemicals). The 8-OH-Gua content was measured by the HPLC-electrochemical detector method after complete digestion as described (10).

Transformation of E. coli DH5α Cells in the Presence of Deoxynucleotide—Competent DH5α cells were prepared as described (26). To 100 μl of the E. coli suspension, 5 μl of the solution containing 1 ng (0.29 fmol) of pMY189 (27) and 25 nmol of a nucleotide was added. The transformation was carried out by the standard method (26).

RESULTS

Induction of Mutations in the Chromosomal lacI Gene by Damaged DNA Precursors—We treated wild type E. coli W3110 with various deoxyribonucleotides, and the frequencies of lacI− and lacO− mutations in the chromosomal DNA were measured. When 50 nmol of either dGTP or dATP was added to the bacteria, the MF observed was very similar to that of the control (no addition of nucleotide) (Table I). On the other hand, the MF was found to be increased when 50 nmol of either 8-OH-dGTP or 2-OH-dATP was added. The relative MFs were increased about 2.5-fold over the control value by the addition of 50 nmol of either 8-OH-dGTP or 2-OH-dATP (Table I). 2-OH-dATP appeared to induce more mutations than 8-OH-dGTP. Moreover, the increases in the MF were dependent on the amount of the nucleotides added (Table I). These results indicate that the two oxidatively damaged nucleotides were mutagenic in vivo, as speculated from the results of in vitro experiments (11, 17). No decrease in the survival ratio (number of colonies on LB plates) was observed by the addition of the damaged nucleotides under the conditions used (data not shown and see below).

Analysis of Mutations—we analyzed the sequences of the lacI gene in the lacI− mutants obtained by the treatments with 50 nmol of nucleotide (89 and 86 cases for 8-OH-dGTP and 2-OH-dATP, respectively). 61 mutants obtained with the con-
control experiments were also analyzed. Table II shows the summary of the sequence analyses. The addition or deletion of the 5′-TGGC-3′ sequence was detected in 84% of the mutants in the control experiments. This type of mutation has been reported as the most frequent mutation in the lacI gene (22, 23, 28, 29). 15% of the mutants contained a single base substitution.

On the other hand, the ratios of the TGGC addition or deletion were decreased in the case of the mutations induced by 8-OH-dGTP. The TGGC mutations were observed in only 22% of the mutants (Table II). Single base substitutions made up 76% of the mutations induced by 8-OH-dGTP. Because the MF was increased 2.4-fold by the addition of 8-OH-dGTP (Table I), it is estimated that the frequency of single base substitutions was increased by 12-fold. The mutation found most frequently in the 8-OH-dGTP-induced mutants was an A → G transversion (Table II). This type of mutation was observed in 69% of the mutant colonics. It was already shown that a DNA polymerase incorporated 8-OH-dGMP opposite A and C in template DNAs in vitro (17) and that the incorporation of 8-OH-dGMP elicits A → G transversions (5, 21). Thus, the present result agrees with the previous reports and indicated that the same event occurs in vivo.

51% of the mutations induced by 2-OH-dATP were single base substitutions (Table II). Because the MF increased 2.5-fold by the addition of 2-OH-dATP (Table I), the single base substitutions were increased 9-fold. G → T A transversions were the mutations detected most frequently (48% of the total mutations). This type of mutation appears to occur by the incorporation of 2-OH-dAMP opposite G residues in DNA (see under "Discussion"). The addition/deletion of the 5′-TGGC-3′ sequence was shared by 48% of the mutants. This value was higher than expected. The 2-OH-AdE residue may be involved in the generation of the TGGC mutations.

The distribution of the mutations induced by the two damaged nucleotides is shown in Fig. 2. There were a few minor hot spots: position 886 for 8-OH-dGTP and positions 782 and 918 for 2-OH-dATP (Fig. 2). These facts suggest that the incorporations of the damaged nucleotides were not uniform. Another interesting feature was the strand preference of the G-C → A-T transversions induced by 2-OH-dATP. Of the 37 G-C → A-T transversions observed, the numbers of mutants containing a G → T transversion and a C → A transversion were 27 and 10, respectively (about 3/1, Fig. 2). This was not caused by an abundance of G residues in the nontranscribed strand (the strand shown in Fig. 2), since G and C bases are similarly present (G/C = 1.06). This result suggests that the 2-OH-dAMP was incorporated preferentially opposite G residues in the nontranscribed strand of the lacI gene. In the case of 8-OH-dGTP, no preferences for the transcribed strand were observed (28/33, nontranscribed/transcribed).

Next we searched for the effects of the 5′- and 3′-flanking bases on the A → C transversions induced by 8-OH-dGTP (Table III). The site where the transversions occurred most frequently was a 5′-TA\A A-3′ sequence, in which the mutated A base is underlined (17 cases). This mutation in the 5′-TA\A A-3′ sequence occurred more than twice as frequently as the mutations in other sequences. Because 5′-TA\A A-3′ (\M = any base) sequences are present at two-thirds to one-half of the frequencies of other sequences, this observed preference can be interpreted more effectively. The frequency of A → C mutations in 5′-GA\A A-3′ (\M = any base) sequences was less than those in other sequences (Table III).

Clearer effects of the nearest neighboring bases were found for the G → T transversions induced by 2-OH-dATP. The G → T mutations occurred at the G residues in 5′-GGG-3′ and 5′-G\G-3′ sequences (14 and 8 cases, Table IV). The G → T mutation in other sites appears to distribute similarly. The G → T mutations were detected frequently at the G residues in the 5′-GG\G-3′ (26 cases) sequences, whereas only one case was detected in the 5′-G\G3′ sequences. Thus, the effects of the 5′-flanking base appear to be quite large in the case of 2-OH-dATP.

| TABLE II |
|-------------------|
| **Spectro of E. coli lacI mutations induced by nucleotides** |
| **Cases found (%)** | Spontaneous | 8-OH-dGTP | 2-OH-dATP |
|-------------------|-------------|-----------|-----------|
| Single base substitution | 9 (15) | 68 (76) | 44 (51) |
| Transition | | | |
| G → C → A-T | 2 (3) | 0 (0) | 2 (2) |
| A-T → G-C | 0 (0) | 1 (1) | 1 (1) |
| Transversion | | | |
| G → C → T-A | 1 (2) | 2 (2) | 37 (43) |
| G → C → C-G | 0 (0) | 1 (1) | 1 (1) |
| A-T → C-G | 6 (10) | 61 (69) | 1 (1) |
| A-T → T-A | 0 (0) | 3 (3) | 2 (2) |
| Single base deletion | 1 (2) | 0 (0) | 0 (0) |
| Single base addition | 0 (0) | 0 (0) | 0 (0) |
| Multibase addition | 0 (0) | 1 (1) | 1 (1) |
| Multibase deletion | 0 (0) | 0 (0) | 0 (0) |
| + TGGC | 42 (69) | 17 (19) | 37 (43) |
| − TGGC | 9 (15) | 3 (3) | 4 (5) |

| a | The TGGC addition is not included. |
| b | The TGGC deletion is not included. |
| c | All of the mutations occurred at positions 621–632 in the lacI gene. |

Mutations Induced by Oxidized Nucleotides in E. coli

Damaged Nucleotides Get into Bacteria—Competent W3110 cells were treated with 32P-labeled 8-OH-dGTP or 2-OH-dATP, and radioactivities in the treated cells were counted after thorough washing and lysis. We observed that 1.3% and 0.3% of the added 8-OH-dGTP and 2-OH-dATP, respectively, were present in cells. Thus, the damaged nucleotides entered E. coli cells. Moreover, these results indicate that 8-OH-dGTP was incorporated into the cells 4.5-fold more than 2-OH-dATP.

We next measured 8-OH-Gua content in chromosomal DNA of 8-OH-dGTP-treated bacteria. 8-OH-Gua was detected by the HPLC-electrochemical detector, a method with high sensitivity. We found that the 8-OH-Gua level of the treated cells was 1.58×10⁶ Gua. We observed that the 8-OH-Gua level of the E. coli treated without 8-OH-dGTP was 0.57×10⁵ Gua. Thus, 8-OH-Gua level of 1.01×10⁵ Gua over the background was induced by the treatment with 8-OH-dGTP, suggesting that the added damaged nucleotide entered bacterial cells and was incorporated by DNA polymerase III. Note that in these experiments, we incubated the bacteria for an additional 60 min (compared with 45 min in mutagenesis experiments) because of the amount of DNA required. We speculate that the ratio of 8-OH-Gua may be decreased by cell division and repair processes during this incubation period. Thus, the 8-OH-Gua content was estimated to be higher than 1.58×10⁵ Gua when the bacteria were transferred onto LB and P-gal plates in mutagenesis experiments.

Cytotoxic Effects of Damaged Nucleotides—In the experiments with W3110, the numbers of E. coli colonies on the LB plates were very similar in all cases (data not shown). Thus, the addition of an oxidatively damaged nucleotide did not appear to decrease the survival ratio under the conditions used. This observation may be the result of a low efficiency of the nucleotide incorporation into the cells. To know whether the incorporation of an oxidatively damaged nucleotide was cytotoxic, 2-OH-dATP or 8-OH-dGTP was introduced into E. coli together with a plasmid containing a selection marker gene, and the survival ratio was measured. We used competent E. coli DH5α.
cells, which are frequently employed for transfection experiments, and a plasmid containing β-lactamase gene (pMY189) (27). Competent DH5α cells were treated with pMY189 together with 109 excess amount (25 nmol) of the damaged nucleotides in 5'-NG-3' sites. We counted the number of transformants on an agar plate containing ampicillin as an indicator of the cytotoxicity. 

Table III

Effects of the nearest neighboring bases on A → C transversions induced by 8-OH-dGTP

| 5'-Flanking base | 3'-Flanking base | Total |
|------------------|------------------|-------|
|                  | G                | A     |
| G                | 0                | 2     | 7    |
| A                | 0                | 5     | 6    |
| T                | 2                | 17    | 19   |
| C                | 8                | 1     | 9    |
| Total            | 10               | 25    | 14   |

Table IV

Effects of the nearest neighboring bases on G → T transversions induced by 2-OH-dATP

| 5'-Flanking base | 3'-Flanking base | Total |
|------------------|------------------|-------|
|                  | G                | A     |
| G                | 14               | 2     | 8    |
| A                | 1                | 0     | 1    |
| T                | 0                | 1     | 1    |
| C                | 1                | 2     | 3    |
| Total            | 16               | 4     | 13   |

Table V

Cytotoxic effects of damaged nucleotides in E. coli

| Nucleotide added | E. coli colony obtained (×104) |
|------------------|------------------------------|
| None             | 11.47 (1.0)                  |
| 25 nmol 8-OH-dGTP| 6.11 (0.5)                   |
| 25 nmol 2-OH-dATP| 5.00 (0.4)                   |
| None             | 6.66 (1.0)                   |
| 25 nmol dGTP     | 7.38 (1.1)                   |
| 25 nmol dATP     | 6.91 (1.0)                   |

a Nucleotides added to a 100-μl suspension of competent E. coli DH5α are shown.

b Ampicillin-resistant colonies obtained by transfection with pMY189. Relative colony numbers are shown in parentheses.

c Three separate experiments.

d Two separate experiments.

3. Discussion

8-OH-dGTP and 2-OH-dATP, two major forms of oxidatively damaged DNA precursors, induced lacI and lacO mutations with nearly equal frequencies (Table I). Taken together with the mutation spectra data (Table II), we estimated that the frequencies of single base substitution mutations were increased by 12- and 9-fold with 8-OH-dGTP and 2-OH-dATP, respectively (see "Results"). Because 8-OH-dGTP got into E. coli 4.5-fold more than 2-OH-dATP, the actual mutagenicity of substitutions of 2-OH-dATP was calculated to be about 3-fold that of 8-OH-dGTP. These results imply that the mutagenic potential of 2-OH-dATP is as important as that of 8-OH-dGTP, when present in the nucleotide pool. Since the formation of the 2-OH-Ade base in monomers by ROS is comparable with that of 8-OH-dGTP, the potential of 2-OH-dATP is as important as that of 8-OH-dGTP, which in vivo can form 2-OH-Ade residues in DNA precursors.

8-OH-dGTP induced A7' → G7' transversions almost exclusively (Table II). This finding can be interpreted as follows. DNA polymerase III incorporated 8-OH-dGMP opposite A residues in the DNA, and the polymerase inserted C opposite the 8-OH-Gua bases during the next round of replication (Fig. 3). Similar results have been obtained by the combination of in vitro DNA synthesis and transfection of the synthesized DNA (5, 21). Thus, the new method appears to be effective for the investigation of the mutational properties of a damaged DNA precursor in vivo.

2-OH-dATP induced G-C → T-A transversions in the lacI gene (Table II). This finding implies that either 2-OH-dAMP was incorporated opposite G, and then dTMP was inserted opposite the incorporated 2-OH-Ade residue during the next round of replication (Fig. 3). Therefore, 8-OH-dATP is suggested to contribute to the mutagenic process in vivo.
opposite C, and then dAMP was inserted opposite the incorporated 2-OH-Ade residue during the next round of replication. Because 2-OH-Ade residues in single-stranded plasmid vectors are “read” as A with more than 99% probability (14), the former explanation is most likely. Thus it is probable that the DNA polymerase III of E. coli incorporated 2-OH-dAMP opposite the G residues in the DNA. This conclusion is in contrast to our previous finding that the mammalian DNA polymerase α, another replicative DNA polymerase, inserts 2-OH-dAMP opposite the C residues in DNA (11). However, a 2-OH-Ade-G pair is formed when the Klenow fragment of E. coli DNA polymerase I inserts dGMP opposite 2-OH-Ade during in vitro DNA synthesis (30, 31). Moreover, an A → C transversion is induced by 2-OH-Ade in plasmid vectors in E. coli (14), whereas this type of mutation is not elicited in simian cells (15). Thus, prokaryotic DNA polymerases may characteristically form the 2-OH-Ade-G pair.

The reasons why the mispairing properties of 2-OH-Ade are dependent upon the DNA polymerases are not clear. One possibility is the difference in hydrophobicities of the active site of each polymerase. 2-OH-Ade forms two tautomers, the 2-hydroxy (enol) and 1,2-dihydro-2-oxo (keto) isomers (see Fig. 1). This equilibrium may be affected by the microenvironment around the base (32, 33). Thus, the difference in the hydrophobicity of the active site may affect the enol-keto equilibrium. This putative shift would have an important effect on the formation of a base pair involving 2-OH-Ade (11, 31).

We transfected competent E. coli DH5α cells with a plasmid containing β-lactamase gene in the presence of 8-OH-dGTP or 2-OH-dATP. The number of transformants on an agar plate containing ampicillin was less than that of the transformants obtained with the plasmid alone (Table V). This effect was not due to the inhibition of transformation by the presence of dNTP and/or the increase in ionic strength because the normal nucleotides did not have any effect (Table V). These results indicate that either damaged nucleotide, 8-OH-dGTP or 2-OH-dATP, was cytotoxic to cells upon incorporation. These effects may be the result of mutations in the β-lactamase gene on the plasmid and/or in other essential gene(s) on the chromosome. Alternatively, extension reaction from an 8-OH-Gua or 2-OH-Ade residue at the 3’-end of a primer may block DNA replication. The other possibility is the replication block during translesional synthesis past these residues in the templates. This possibility is unlikely for 2-OH-Ade because the oxidized adenine in double-stranded DNA does not induce the replication block (14).

It is very important that 2-OH-dATP elicited the G-C → T-A transversion, which is one of the mutations frequently induced by ROS. To date, this mutation is thought to be mediated by the formation of 8-OH-Gua in the DNA. However, the formation of 2-OH-dATP in the nucleotide pool will elicit this kind of mutation in bacteria, as shown in this study (Table II). In addition, the G-C → T-A transversion occurs by a lipid peroxidation system without an increase in the formation of 8-OH-Gua in DNA (34). Thus, the G-C → T-A transversion and other mutations appear to occur by a variety of pathways and not just by a single DNA lesion.

The MutT protein hydrolyzes 8-OH-dGTP to produce the cognate monophosphate (17). This function prevents mutations by the damaged nucleotide. Thus, in mutT strains, 8-OH-dGTP will induce more mutations than observed in this study. It is possible that a MutT-like activity may eliminate 2-OH-dATP, which was as mutagenic as 8-OH-dGTP (Table I). Further studies will be necessary to determine the involvement of the putative MutT-like activity in the prevention of mutations induced by various oxidized deoxyribonucleoside triphosphates.

One of our major objectives was to establish a new method to evaluate the mutagenicity of a damaged DNA precursor (deoxyribonucleoside 5'-triphosphate) in vivo. To our knowledge, the mispairing properties of an oxidatively damaged DNA precursor have been evaluated by in vitro DNA polymerase reactions and by mutagenesis experiments using vector DNA in which the oxidized precursor is incorporated by in vitro DNA synthesis (5, 11, 17, 19–21). It should be emphasized that the MutT-like activity is a factor in the determination of the mutagenicity of damaged nucleotides in the in vivo method. Thus, our new approach will effectively complement the in vitro method. One may think that this new method resembles experiments in which modified nucleosides are added to the culture medium (35). In this type of experiment, a modified nucleoside must be converted to the cognate triphosphate prior to the incorporation into DNA. The total efficiency of the kination reactions is very different, depending upon the structure of the modified nucleoside. However, our approach eliminates the effects of differences in the enzymatic kination reaction efficiency and directly evaluates the mutagenicity of damaged nucleoside triphosphates.

Another major objective was to investigate the mutational properties of 2-OH-dATP, which is produced by ROS. We demonstrated that 2-OH-dATP was as mutagenic as 8-OH-dGTP (Table I). Moreover, 8-OH-Gua and 2-OH-Ade in double-stranded DNA induce mutations with similar frequencies in E. coli and mammalian cells (5–8, 14, 15). These results indicate that 2-OH-Ade is an important oxidative lesion. Furthermore, 2-OH-Ade in DNA appears to be repaired less efficiently in bacteria, as shown in this study (Table II). In addition, the G-C → T-A transversion occurs by a lipid peroxidation system without an increase in the formation of 8-OH-Gua in DNA (34). Thus, the G-C → T-A transversion and other mutations appear to occur by a variety of pathways and not just by a single DNA lesion.

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