Metabolic Fate of Oxidized Guanine Ribonucleotides in Mammalian Cells[†]

Hiroshi Hayakawa,*[,‡] Anders Hofer,[§] Lars Thelander,[§] Shigetaka Kitajima,[||] Yong Cai,[||] Satoru Oshiro,[||] Hiroyuki Yakushiji,[⊥] Yusaku Nakabeppu,[⊥] Michihiko Kuwano,[‡] and Mutsuo Sekiguchi[▽]

Department of Biochemistry, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan, Department of Medical Biochemistry and Biophysics, Umeå University, S-901 87 Umeå, Sweden, Department of Biochemical Genetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo 113-0034, Japan, Department of Biochemistry, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan, and Department of Biology, Fukuoka Dental College, Fukuoka 814-0193, Japan

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ABSTRACT: 8-Oxo-7,8-dihydroguanine- (8-oxoguanine-) containing nucleotides are generated in the cellular nucleotide pool by the action of oxygen radicals produced during normal cellular metabolism. We examined the interconversion and metabolic fate of 8-oxoguanine-containing ribonucleotides in mammalian cells. (1) 8-OxoGTP can be generated not only by direct oxidation of GTP but also by phosphorylation of 8-oxoGDP by nucleotide diphosphate kinase, and the 8-oxoGTP thus formed can serve as a substrate for RNA polymerase II to induce transcription errors. (2) MTH1 protein carrying intrinsic 8-oxo-dGTPase activity has the potential to hydrolyze 8-oxoGTP to 8-oxoGMP, thus preventing misincorporation of 8-oxoguanine into RNA. 8-OxoGMP, the degradation product, cannot be reutilized, since guanylate kinase, which has the potential to phosphorylate both GMP and dGMP, is inactive on 8-oxoGMP. (3) Ribonucleotide reductase, which catalyzes reduction of four naturally occurring ribonucleoside diphosphates, cannot convert 8-oxoguanine-containing ribonucleotide to the deoxyribonucleotide. This step appears to serve as a gatekeeper to prevent formation of mutagenic substrates for DNA synthesis from oxidized ribonucleotides.

Oxygen radicals are produced through normal cellular metabolism, and formation of these radicals is further enhanced by ionizing radiation and by various chemicals *(1)*. The oxygen radicals attack nucleic acids and various modified bases in DNA are generated. Among them, 8-oxo-7,8-dihydroguanine (8-oxoguanine)[1] is the most abundant, and appears to play critical roles in carcinogenesis and in aging *(2, 3)*. 8-Oxoguanine can pair with both cytosine and adenine during DNA synthesis, and as a result G•C to T•A transversion can be induced *(4, 5)*. Oxidation of guanine also occurs in the cellular nucleotide pool. The 8-oxo-dGTP thus formed is a potent mutagenic substrate for DNA synthesis since it can be incorporated into DNA opposite adenine as well as cytosine, at almost equal efficiency *(6)*. Studies using *Escherichia coli* mutator mutants revealed that cells possess elaborate mechanisms that prevent mutations caused by oxidation of the guanine base, in both DNA and free nucleotide forms *(7)*. Similar mechanisms appear to function in mammalian cells to prevent the occurrence of mutations.

The metabolic fate of 8-oxoguanine-containing deoxyribonucleotides in mammalian cells has been studied extensively. 8-Oxo-dGTP is hydrolyzed to 8-oxo-dGMP by the action of MTH1 protein, a mammalian counterpart of the *E. coli* MutT protein *(8, 9)*. Guanylate kinase, which phosphorylates GMP and dGMP to the corresponding nucleoside diphosphates *(10)*, is inactive on 8-oxo-dGMP, thus preventing reutilization of the MTH1 cleavage product *(11)*. 8-Oxo-dGMP is rapidly dephosphorylated, yielding 8-oxodeoxyguanosine, a form readily excretable to the cell exterior. 8-Oxodeoxyguanosine is present in mammalian urine, and it has been proposed that urinary 8-oxodeoxyguanosine can serve as a biomarker for oxidative stress *(12)*.

Taddei et al. *(13)* found that 8-oxoguanine can be misincoporated into RNA, causing transcriptional error. They further demonstrated that the *E. coli* MutT protein counteracts this event by degrading 8-oxoguanine-containing ribonucleoside triphosphate to the monophosphate. We asked if this scheme can be applied to the information transfer machinery in mammalian cells. Among three types of RNA polymerases functioning in eukaryotic cells, polymerase II is responsible for synthesis of messenger RNA and it was of interest to determine if this enzyme can utilize 8-oxoGTP as a substrate for RNA synthesis. Mammalian cells should be equipped with a mechanism to eliminate 8-oxoGTP from the RNA

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* To whom correspondence should be addressed: Tel 81-92-642-6100; Fax 81-92-642-6203; E-mail hiroshi@mailserver.med.kyushu-u.ac.jp.

[‡] Faculty of Medicine, Kyushu University.

[§] Umeå University.

[||] Tokyo Medical and Dental University.

[⊥] Medical Institute of Bioregulation, Kyushu University.

[△] Fukuoka Dental College.

[1] Abbreviations: 8-oxoguanine, 8-oxo-7,8-dihydroguanine; 8-oxoGTP, 8-oxo-7,8-dihydroguanosine 5′-triphosphate; 8-oxo-dGTP, 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate; 8-oxo-dGTPase, 8-oxo-7,8-dihydrodeoxyguanosine triphosphatase; 8-oxoGMP, 8-oxo-7,8-dihydroguanosine 5′-monophosphate; 8-oxo-dGMP, 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-monophosphate; 8-oxoGDP, 8-oxo-7,8-dihydroguanosine 5′-diphosphate; 8-oxo-dGDP, 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-diphosphate; DTT, dithiothreitol; TLC, thin-layer chromatography.

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precursor pool. MTH1 protein, a homologue of MutT protein, is one candidate, but these two proteins have different substrate specificities *(6, 14)*. Thus, a detailed study of the activity of MTH1 protein is required.

The pool size of ribonucleotides is hundreds of times larger than that of deoxyribonucleotides *(15)*. This means that a significantly larger amount of 8-oxoguanine-containing ribonucleotides are probably produced, compared to deoxyribonucleotide counterparts in mammalian cells. Since ribonucleotide reductase, which is responsible for the conversion of ribonucleotides to deoxyribonucleotides, has a relatively broad substrate specificity, 8-oxoguanine-containing deoxyribonucleotide may perhaps be produced by reduction of the corresponding ribonucleotide. This possibility can be examined by determining the substrate specificity of reductase enzyme prepared from mammalian cells.

EXPERIMENTAL PROCEDURES

Chemicals and Enzymes. 8-OxoGTP and the corresponding diphosphate and monophosphate were prepared as described *(11)*. RNA polymerase II was purified from calf thymus according to Hodo and Blatti *(16)*. A homogeneous preparation of human MTH1 protein was obtained as described by Yakushiji et al. *(17)*. Mouse ribonucleotide reductase R1 and R2 proteins were prepared as described *(18)*. Bovine guanylate kinase and P1 nuclease were purchased from Sigma and Boehringer Mannheim, respectively. A cell-free extract was prepared from cultured Jurkat cells (a human T-cell leukemia cell line), as described *(11)*.

In Vitro Transcription. α-^{32}P-Labeled GTP or 8-oxoGTP (500 pmol) was incubated with various amounts of a purified preparation of calf thymus RNA polymerase II in a reaction mixture (50 μL) containing 6 mM $MnSO_4$, 100 mM $(NH_4)_2SO_4$, 0.02 mM EDTA, 0.1 mM ATP, 0.1 mM UTP, 0.1 mM CTP, 5% glycerol, 0.4 mg/mL heat-denatured calf thymus DNA, and 50 mM Tris-HCI (pH 7.9). The reaction was run at 37 °C for 30 min and terminated by addition of 250 μL of 10% trichloroacetic acid. The acid-insoluble material was collected by centrifugation and washed twice with 1 N HCl−5% pyrophosphate and then with ethanol. Radioactivity was determined by a liquid scintillation counter.

Product Analysis. The acid-insoluble material from in vitro transcription was dissolved in 20 μL of 50 mM sodium acetate (pH 5.3) and treated with 0.2 mg/mL P1 nuclease at 37 °C for 2 h. The digest was spotted onto a TLC plate, PEI-cellulose F (Merck), and developed in 1 M LiCl. Radioactive spots were monitored by a Fujix BSA2000 image analyzer.

GTPase Assay. α-^{32}P-Labeled GTP or 8-oxo-GTP (200 pmol) was incubated with various amounts of purified MTH1 protein in a mixture (10 μL) containing 4 mM $MgCl_2$, 40 mM NaCl, 80 μg/mL bovine serum albumin, 8 mM DTT, 10%(v/v) glycerol, and 20 mM Tris-HCl (pH 8.0). The reaction was run at 30 °C for 10 min and terminated by adding EDTA. An aliquot of the reaction mixture was spotted onto a TLC plate and developed in 1 M LiCl. Amounts of nucleoside monophosphate produced were quantified, as described above.

Guanylate Kinase Assay. The reaction mixture (10 μL) containing 0.1 M Tris-HCl (pH 8.0), 0.25 M KCl, 20 mM $MgCl_2$, 5 mM ATP, 8 nmol of either α-^{32}P-labeled GMP, or 8-oxoGMP and various amounts of enzyme was incubated at 37 °C for 5 min *(19)*. The reaction was terminated by adding EDTA and the mixture was applied to a TLC plate. Amounts of nucleoside diphosphate formed were quantified, as described above.

Ribonucleotide Reductase Assay. Aliquots (250 pmol) of α-^{32}P-labeled GDP or 8-oxoGDP were incubated in a reaction mixture (50 μL) containing 0.05 M KCl, 6.4 mM $MgCl_2$, 0.02 mM $FeCl_3$, 10 mM DTT, 8.6 mM Hepes, 22 mM Tris-HCl (pH 7.6), and a preparation of recombinant mouse ribonucleotide reductase, consisting of 40 μg/mL Rl subunit and 15 μg/mL R2 subunit proteins. Various nucleotides were included as effectors. The reaction was run at 37 °C for 30 min and terminated by heating for 5 min. After removal of the precipitate by centrifugation, 2.5 μL of 1 M $(NH_4)HCO_3$ (pH 8.9) were added to the supernatant to give a 50 mM concentration of ammonium bicarbonate. The mixture was applied onto a borate column (1.3 cm^3) equilibrated with 50 mM $(NH_4)HCO_3$−15 mM $MgCl_2$ (pH 8.9) *(20)*. The column was eluted with the same buffer, and the first two 1-mL fractions, which contain deoxyribonucleotides, were collected. The column was further processed with 6 mL of 0.1 M sodium borate (pH 8.9) to elute ribonucleotides. Amounts of deoxyribonucleoside diphosphates produced were determined by dividing the radioactivity in the first two-1 mL fractions by the total radioactivity applied.

Other Procedures. Protein concentration was determined by use of Bio-Rad protein assay kits with bovine serum albumin as a standard *(21)*. Other procedures used were as described previously *(11)*.

RESULTS

Misincorporation of 8-Oxoguanine into RNA. Mammalian cells contain potent nucleoside diphosphate kinase activity that phosphorylates various nucleoside diphosphates to the corresponding nucleoside triphosphates *(15)*. When 8-oxoGDP was incubated with an extract of human Jurkat cells in the presence of ATP, it was rapidly converted to 8-oxoGTP *(11)*. 8-OxoGTP can also be produced by direct oxidation of GTP.

Once 8-oxoGTP is formed, it may possibly serve as a substrate for RNA synthesis. To examine this possibility, an in vitro transcription experiment was done with a purified preparation of calf thymus RNA polymerase II. When α-^{32}P-labeled 8-oxoGTP was incubated with three types of ribonucleotide substrates in the reaction mixture, a low but significant amount of radioactivity was incorporated into the acid-insoluble material. The rate of incorporation of 8-oxoguanine was about 2% that of guanine (Figure 1A). To confirm that 8-oxoguanine is present in RNA, the reaction product was analyzed. The acid-insoluble material was digested with P1 nuclease and the resulting products were analyzed. As shown in Figure 1B, the reaction performed with 8-oxoGTP yielded a product that can be identified as 8-oxoGMP (for line 3) while that with GTP produced one identical with GMP (for line 1). Thus, 8-oxoguanine is incorporated into mRNA, by mammalian RNA polymerase II.

Cleavage of 8-OxoGTP by MTH1 Protein. To counteract transcriptional errors caused by oxidative damage, mammalian cells probably have a mechanism to eliminate

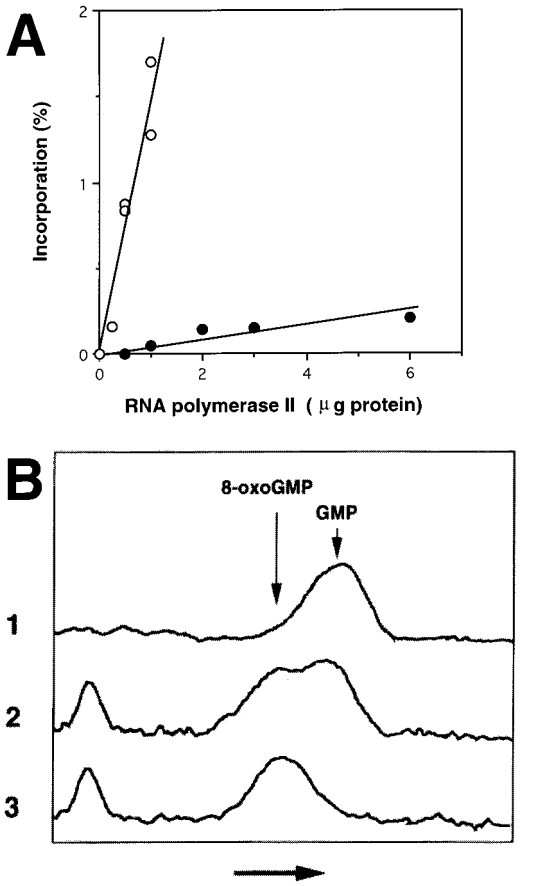

FIGURE 1: Incorporation of 8-oxoguanine into RNA by calf thymus RNA polymerase II. (A) Incorporation of α-^{32}P-labeled materials into the acid-insoluble fraction. α-^{32}P-Labeled nucleoside triphosphate was incubated with various amounts of a purified preparation of calf thymus RNA polymerase II at 37 °C for 30 min. For obtaining relative rate of incorporation (%), radioactivity in the acid-insoluble fraction was divided by radioactivity applied to the reaction mixture. (○), GTP; (●) 8-oxoGTP. (B) Analysis of transcripts. The acid-insoluble materials were digested with P1 nuclease and were applied to TLC. Profiles of radioactive distribution were obtained by BAS2000 image analyzer. The horizontal arrow indicates the direction of development. Line 1, a transcript formed with labeled GTP; line 2, a mixture of samples for 1 and 3; line 3, a transcript formed with labeled 8-oxoGTP.

8-oxoGTP from the RNA precursor pool. One of the candidates for this is MTH1 protein, which has the capacity to cleave 8-oxo-dGTP, the deoxyribonucleotide counterpart. When 8-oxoGTP was incubated with a purified preparation of human MTH1 protein, it was rapidly converted to 8-oxoGMP. As shown in Figure 2, a large amount of 8-oxoGTP was hydrolyzed when increasing amounts of MTH1 protein were present. Under the same condition, GTP was not degraded. It should be noted, however, that the rate of cleavage of 8-oxoGTP by MTH1 protein was 50 times lower than that for 8-oxo-dGTP. This is in contrast to *E. coli* MutT protein which hydrolyzes the two types of oxoguanine-containing nucleotiude triphosphates with much the same efficiency *(13)*.

8-OxoGMP, produced by the action of MTH1 protein, cannot be reutilized, since mammalian cells apparently have no enzyme activity to phosphorylate 8-oxoGMP. This was evident when a crude extract prepared from human cells was used, which contains potent guanylate kinase and nucleoside diphosphate kinase activities (Figure 3), and also with a

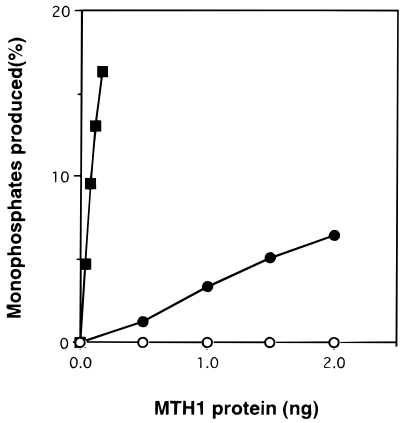

FIGURE 2: Hydrolysis of 8-oxoGTP, GTP, and 8-oxo-dGTP by human MTH1 protein. α-^{32}P-Labeled nucleoside triphosphates were incubated with various amounts of a purified preparation of human MTH1 protein (0.5−2.0 ng) in a reaction mixture (10 μL). The reaction was run at 30 °C for 10 min, and terminated by the addition of EDTA. (○) GTP; (●) 8-oxoGTP; (■) 8-oxo-dGTP.

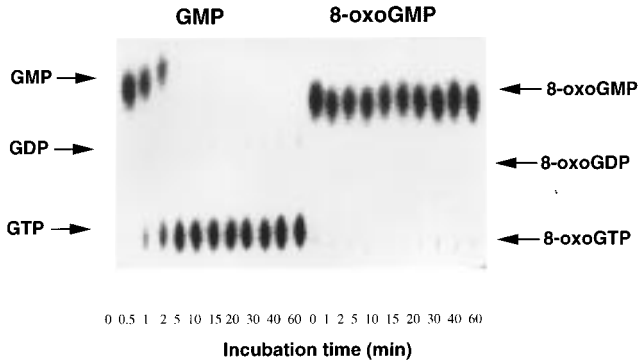

FIGURE 3: Phosphorylation of guanosine monophosphates by a Jurkat cell-free extract. α-^{32}P-Labeled GMP or 8-oxoGMP (8 nmol) was incubated with 2.8 μg of protein, Jurkat cell-free extract, in a reaction mixture (10 μL) containing 0.1 M Tris-HCl, pH 8.0, 0.25 M KCl, 20 mM $MgCl_2$, and 5 mM ATP. After incubation at 37 °C for the times indicated (minutes), the reaction was terminated by the addition of EDTA.

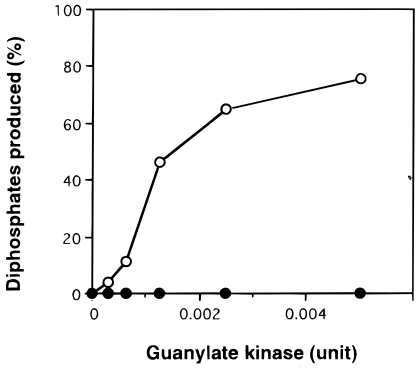

FIGURE 4: Substrate specificity of bovine guanylate kinase. α-^{32}P-Labeled GMP or 8-oxoGMP (8 nmol) was incubated with various amounts of bovine guanylate kinase in a reaction mixture (10 μL) at 37 °C for 5 min. The reaction was terminated by the addition of EDTA and the products were analyzed by TLC. (○) GMP; (●) 8-oxoGMP.

purified guanylate kinase preparation (Figure 4). Phosphorylation of 8-oxoGMP never occurred but GMP rapidly converted to GDP. This would facilitate elimination of 8-oxoguanine-containing ribonucleotides from the precursor pool for RNA synthesis.

Table 1: Action of Ribonucleotide Reductase on Two Types of Guanine Nucleotides[a]

substrate	enzyme	effector(s)	reduction product (pmol)	reduction (%)
GDP	−	2.0 mM ATP, 0.5 mM TTP	<0.1	0
	+	2.0 mM ATP, 0.5 mM TTP	173.0	69
8-oxoGDP	−	2.0 mM ATP, 0.5 mM TTP	<0.1	0
	+	2.0 mM ATP, 0.5 mM TTP	<0.1	0
	+	2.0 mM ATP, 0.5 mM dGTP	<0.1	0
	+	2.0 mM ATP	<0.1	0
	+	0.1 mM dATP	<0.1	0

[a] α-^{32}P-labeled GDP or 8-oxoGDP (250 pmol) was incubated with a purified preparation of ribonucleotide reductase in the presence of various combinations of effectors.

Blockage for the Deoxyribonucleotide Pool. Enzymatic conversion of ribonucleotides to deoxyribonucleotides occurs at the level of nucleoside diphosphate, and ribonucleotide reductase, the enzyme responsible, has a relatively broad substrate specificity. Four types of naturally occurring ribonucleotides, namely, ADP, GDP, CDP, and UDP, are converted to the corresponding deoxyribonucleotides by a single species of reductase enzyme *(15)*. Hence, it was initially assumed that 8-oxoguanine-containing ribonucleotides may be converted by this enzyme to deoxyribonucleotide forms.

We first attempted to examine this possibility using a crude enzyme preparation from human cells *(11)*. However, it was difficult to examine the reductase activity with such a preparation, which contained potent nucleoside diphosphate kinase activity; incubation of 8-oxoGDP in the presence of ATP resulted in formation of 8-oxoGTP.

To overcome this difficulty, we used a homogeneous reductase enzyme. The enzyme carries a distinct reductase activity and is free of other enzyme activity. The result is summarized in Table 1. When GDP was incubated with this enzyme preparation, it was converted to dGDP. However, under the same conditions, conversion of 8-oxoGDP to 8-oxo-dGDP did not occur. Addition of various combinations of nucleotide effectors known to modulate the substrate specificity of the enzyme *(22)* yielded no positive effect. On the basis of findings in our experiments, 8-oxoguanine-containing deoxyribonucleotide cannot be produced through reduction of 8-oxoGDP.

DISCUSSION

Taddei et al. *(13)* reported that 8-oxoGTP can serve as a substrate for *E. coli* RNA polymerase. With *E. coli* DNA as the template, 8-oxoguanine was incorporated into RNA at a rate 10% that of guanine. This misincorporation could be prevented by MutT protein, which hydrolyzes 8-oxoGTP to 8-oxoGMP, a form not usable for RNA synthesis. The present study was undertaken to see if a similar mechanism functions in mammalian cells for reducing frequency of transcriptional errors.

In in vitro transcription by mammalian RNA polymerase II, 8-oxoguanine could be misincorporated into RNA; however, the rate of incorporation of 8-oxoguanine was only 2% that of guanine. Thus, the RNA polymerase responsible for synthesis of major transcripts in mammalian cells is more selective in discriminating unfavorable nucleotides from the substrate, as compared with the prokaryotic polymerase.

Human MTH1 protein is capable of hydrolyzing 8-oxoGTP, but the potential is about 2% that for 8-oxo-dGTP.

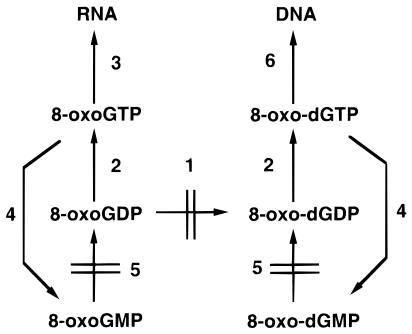

FIGURE 5: Interconversion of 8-oxoguanine-containing nucleotides in mammalian cells. This scheme is based on the results of an earlier study *(11)* plus the present data. (1) Ribonucleotide reductase; (2) nucleoside diphosphate kinase; (3) RNA polymerase; (4) MTH1 protein; (5) guanylate kinase; (6) DNA polymerase. Double bars represent a block of the reaction.

This is in contrast to the capacity of *E. coli* MutT protein, which degrades 8-oxoGTP as efficiently as does 8-oxo-dGTP. In this regard, it is possible that another enzyme takes a charge to hydrolyzed 8-oxoGTP in mammalian cells. Several enzymic activities that hydrolyze 8-oxoGTP and generate 8-oxoGMP were detected in MTH1 knock-out mice liver extract. However, no 8-oxoguanine-specific 8-oxoGTPase has been found (unpublished results). 8-OxoGMP thus produced is not phosphorylated to 8-oxoGDP, whereas 8-oxoGDP can be easily phosphorylated to 8-oxoGTP. These results are not surprising but convincing. The conversion of monophosphates to diphosphates is catalyzed by kinases that are specific for each base, while the phosphorylation of diphosphates to triphosphates is catalyzed by a sole enzyme, nucleoside diphosphate kinase. The powerful and ubiquitous enzyme shows no preference for either ribose or deoxyribose nor for purines or pyrimidines, including a large variety of synthetic analogues *(15)*.

8-Oxo-dGTP is a mutagenic substrate for DNA synthesis, and the principal function of the MutT as well as of the MTH1 protein is to eliminate 8-oxo-dGTP from the DNA precursor pool *(6, 23)*. 8-Oxo-dGTP can be formed by direct oxidation of dGTP or by phosphorylation of 8-oxo-dGDP *(11)*. Since conversion of ribonucleotides to deoxyribonucleotides occurs at the level of nucleoside diphosphate, it is important to determine if 8-oxoGDP is affected by actions of ribonucleotide reductase. We found that a preparation of mouse recombinant ribonucleotide reductase did not reduce 8-oxoGDP but did convert GDP to dGDP. The substrate specificity of the reductase enzyme is modulated by binding of effector nucleotides to allosteric control sites of the protein. When ATP is bound to the sites, reduction of UDP and CDP is favored, and binding of TTP and dGTP to the same sites stimulates reduction of GDP and ADP *(22)*. The addition of combinations of these nucleotides to the reaction mixture did not lead to the reduction of 8-oxoGDP. Thus, 8-oxo-dGDP apparently cannot be supplied through the reduction of oxidized ribonucleotide. The ribonucleotide reductase may serve as a gatekeeper to prevent influx of potent genotoxic substrates from the oxidized ribonucleotide pool (Figure 5).

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REFERENCES

1. Ames, B. N., and Gold, L. S. (1991) *Mutat. Res. 250*, 3−16.
2. Kasai, H., Crain, P. F., Kuchino, Y., Nishimura, S., Ootsuyama, A., and Tanooka, H. (1986) *Carcinogenesis 7*, 1849−1851.
3. Bessho, T., Tano, K., Kasai, H., and Nishimura, S. (1992) *Biochem. Biophys. Res. Commun. 188*, 372−378.
4. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) *Nature 349*, 431−434.
5. Moriya, M. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 1122−1126.
6. Maki, H., and Sekiguchi, M. (1992) *Nature 355*, 273−275.
7. Tajiri, T., Maki, H., and Sekiguchi. M. (1995) *Mutat. Res. 336*, 257−267.
8. Sakumi, K., Furuichi, M., Tsuzuki, T., Kakuma, T., Kawabata, S., Maki, H., and Sekiguchi, M. (1993) *J. Biol. Chem. 268*, 23524−23530.
9. Furuichi, M., Yoshida, M. C., Oda, H., Tajiri, T., Nakabeppu, Y., Tsuzuki, T., and Sekiguchi, M. (1994) *Genomics 24*, 485−490.
10. Miech, R. P., and Parks, R. E., Jr. (1965) *J. Biol. Chem. 240*, 351−357.
11. Hayakawa, H., Taketomi, A., Sakumi, K., Kuwano, M., and Sekiguchi, M. (1995) *Biochemistry 34*, 89−95.
12. Shigenaga, M. K., Gimeno, C. J., and Ames, B. N. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 9697−9701.
13. Taddei, F., Hayakawa, H., Bouton, M.-F., Cirinesi, A.-M., Matic, I., Sekiguchi, M., and Radman M. (1997) *Science 278*, 128−130.
14. Mo, J.-Y., Maki, H., and Sekiguchi, M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 11021−11025.
15. Kornberg, A., and Baker, T. A. (1992) *DNA replication*, 2nd ed., W. H. Freeman and Co., New York.
16. Hodo, H. G., and Blatti, S. P. (1977) *Biochemistry 16*, 2334−2343.
17. Yakushiji, H., Maraboeuf, F., Takahashi, M., Deng, Z.-S., Kawabata, S., Nakabeppu, Y., and Sekiguchi, M. (1997) *Mutat. Res. 384*, 181−194.
18. Davis, R., Thelander, M., Mann, G. J., Behravan, G., Soucy, F., Beaulieu, P., Lavallee, P., Gräslund. A., and Thelander, L. (1994) *J. Biol. Chem. 269*, 23171−23176.
19. Oeschger, M. P., and Bessman, M. J. (1966) *J. Biol. Chem. 241*, 5452−5460.
20. Shewach, D. S. (1992) *Anal. Biochem. 206*, 178−182.
21. Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.
22. Eriksson, S., Thelander, L., and Åkerman, M. (1979) *Biochemistry 18*, 2948−2952.
23. Sekiguchi, M. (1996) *Genes Cells 1*, 139−145.

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