Human MTH1 protein hydrolyzes the oxidized ribonucleotide, 2-hydroxy-ATP

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ABSTRACT

The human nucleotide pool sanitization enzyme, MTH1, hydrolyzes 2-hydroxy-dATP and 8-hydroxy-dATP in addition to 8-hydroxy-dGTP. We report here that human MTH1 is highly specific for 2-hydroxy-ATP, among the cognate ribonucleoside triphosphates. The pyrophosphatase activities for 8-hydroxy-GTP, 2-hydroxy-ATP and 8-hydroxy-ATP were measured by high-performance liquid chromatography. The kinetic parameters thus obtained indicate that the catalytic efficiencies of MTH1 are in the order of 2-hydroxy-dATP > 2-hydroxy-ATP > 8-hydroxy-dGTP > 8-hydroxy-dATP >> dGTP > 8-hydroxy-GTP > 8-hydroxy-ATP. Notably, MTH1 had the highest affinity for 2-hydroxy-ATP among the known substrates. ATP is involved in energy metabolism and signal transduction, and is a precursor in RNA synthesis. We suggest that the 2-hydroxy-ATP hydrolyzing activity of MTH1 might prevent the perturbation of these ATP-related pathways by the oxidized ATP.

INTRODUCTION

Endogenous oxidation of nucleotides by reactive oxygen species (ROS) appears to induce spontaneous mutations, aging and various diseases, including cancer and neurodegeneration (1,2). Bacterial MutT hydrolyzes 8-hydroxy-dGTP (8-OH-dGTP), a major oxidation product of dGTP, which is incorporated opposite adenine and cytosine by DNA polymerases (3–6). Since the genetic mutation rate in a *mutT*-deficient strain is increased by up to 1000-fold as compared to the wild-type (7), it is considered that 8-OH-dGTP is a major source of spontaneous mutations caused by endogenous ROS, and that MutT suppresses spontaneous errors in DNA replication. Previously, it was shown that 8-hydroxy-GTP (8-OH-rGTP) is degraded by the purified bacterial MutT protein as efficiently as 8-OH-dGTP (8). 8-OH-rGTP is misincorporated opposite adenine residues on a poly(dA-dT) template at one-fifth the rate of UTP by

E.coli RNA polymerase. Since a *mutT*-deficient strain gives a 30-fold increase in the phenotypic *lacZ* reversion rate in non-dividing cells under aerobic conditions, 8-OH-dGTP is considered to be a critical source of transcription errors. Thus, MutT appears to suppress errors efficiently during replication and transcription.

The human MutT homologue, MTH1, hydrolyzes 8-OH-dGTP *in vitro* and suppresses the mutator phenotype of *E.coli mutT*-deficient cells (9,10). However, Hayakawa *et al.* showed that MTH1 hydrolyzes 8-OH-rGTP at only 2% of the rate of 8-OH-dGTP (11). Thus, the involvement of MTH1 in RNA pool sanitization in human cells has been questionable.

We have previously shown that MTH1 hydrolyzes the oxidized adenine nucleotides, 2-hydroxy-dATP (2-OH-dATP) and 8-hydroxy-dATP (8-OH-dATP), in addition to 8-OH-dGTP (12). In particular, 2-OH-dATP is the best substrate for the MTH1 protein among these nucleotides, and is hydrolyzed with higher affinity than 8-OH-dGTP. It is also known that 2-hydroxyadenine incorporated into DNA is a good substrate for the human base excision repair enzyme, the MYH protein (13). Since 2-OH-dATP is produced efficiently by ROS-treatment of dATP, and is more mutagenic than 8-OH-dGTP *in vivo* (5,14), the human MTH1 protein appears to eliminate 2-OH-dATP, the potential endogenous mutagen, from the DNA precursor pool. This characteristic function of the MTH1 protein, which differs from the bacterial MutT protein, is supported by recent studies correlating the structures and activities of these proteins (15,16).

ATP is an important material involved in RNA synthesis, energy metabolism and signal transduction, and many ATP analogues are known to inhibit these pathways (17–20). In addition, the amount of oxidized ATP(s) is possibly larger as compared with 8-OH-rGTP, because the ATP pool size in human cells is the largest ($3.12 nmol/10^6$ cells) among the ribonucleoside triphosphate pools, and is ~5-fold larger than the GTP pool size (21). We speculate that the human MTH1 protein may hydrolyze oxidized ATP(s) as well as the cognate deoxyribonucleotides and may prevent the perturbation of ATP-related pathways caused by damaged ATP(s). In this paper, we describe how the human MTH1 protein hydrolyzed

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2-hydroxy-ATP (2-OH-rATP) with an efficiency similar to that for 8-OH-rGTP, and with the highest affinity among the known substrates.

MATERIALS AND METHODS

Materials

The human MTH1 protein was purified from an *E.coli* over-expression system (22). ATP, AMP, GTP and GMP were purchased from Sigma. GDP, Ado and Guo were purchased from Yamasa. The authentic 8-hydroxy-Guo was prepared from 8-bromo-Guo, as described (23). The authentic 8-hydroxy-Ado and 2-hydroxy-Ado were chemically synthesized (23,24). Nucleotide detection and quantification were performed with a Hewlett-Packard 1040M HPLC Detection System.

Treatment of ribonucleotides and ribonucleosides with Fe(II)–EDTA

The ROS-treatments of ATP, GTP, AMP, GMP, Ado and Guo were carried out by a procedure similar to that described previously (14). Aqueous buffered (10 mM sodium phosphate, pH 7.4) solutions of 1 mM nucleotides were vigorously shaken with 5 mM ferrous sulfate and 5 mM EDTA at room temperature for 30 min. The ATP reaction mixture was immediately subjected to anion-exchange high-performance liquid chromatography (HPLC) using a TSK-GEL DEAE-2SW column (4.6 × 250 mm; Tosoh) with an isocratic elution consisting of 50 mM sodium phosphate buffer, pH 7.0, and 15 mM EDTA at a flow rate of 1.2 ml/min, and with UV monitoring. The GTP reaction mixture was fractionated by anion-exchange HPLC as before with an isocratic elution consisting of 15 mM sodium phosphate buffer, pH 7.0, and 15 mM EDTA at a flow rate of 1.2 ml/min. The AMP reaction mixture was fractionated by anion-exchange HPLC as before with an isocratic elution consisting of 25 mM sodium dihydrogenphosphate at a flow rate of 1.2 ml/min. The GMP reaction mixture was subjected to reverse-phase HPLC using a YMC-Pack ODS-AM column (4.6 × 250 mm, YMC) with an isocratic elution by a 30% concentration of solution A (12.5 mM citric acid, 25 mM sodium acetate and 10 mM acetic acid) at a flow rate of 1.0 ml/min. The Ado reaction mixture was fractionated by reverse-phase HPLC using an Ultrasphere ODS 5-μm column (4.6 × 250 mm; Beckman) with an isocratic elution by 5% methanol at a flow rate of 1.0 ml/min. The Guo reaction mixture was fractionated by reverse-phase HPLC using a YMC-Pack ODS-AM column with an isocratic elution consisting of solution A, 30 mM sodium hydroxide and 5% methanol at a flow rate of 1.0 ml/min.

Preparation of 2-OH-rATP

2-OH-rATP was prepared by vigorous shaking of solutions of ATP (10 mg) with Fe(II)–EDTA (40 mM ferrous sulfate and 40 mM EDTA) in 1.5 ml of 50 mM sodium phosphate buffer (pH 7.4) under air at room temperature for 30 min. 2-OH-rATP was separated by anion-exchange HPLC using a TSK-GEL DEAE-2SW column with an isocratic elution consisting of 75 mM sodium phosphate buffer, pH 7.0, and 20% acetonitrile at a flow rate of 0.6 ml/min, and by monitoring of the UV spectra. The 2-OH-rATP fraction was further purified by reverse-phase HPLC using a YMC-Pack ODS-AM column with isocratic elution by solution A containing 45 mM sodium

hydroxide at a flow rate of 0.8 ml/min. The 2-OH-rATP fraction was injected into a TSK-GEL DEAE-2SW column with an isocratic elution by 250 mM triethylammonium hydrogen-carbonate at a flow rate of 0.6 ml/min. The triethylammonium hydrogencarbonate was removed from the preparations by repeated co-evaporation (five times) with water and methanol (1:1). The nucleoside obtained by dephosphorylation with bacterial alkaline phosphatase (Sigma) was identified as 2-hydroxy-Ado by its spectroscopic and chromatographic behaviors on reverse-phase HPLC using a YMC-Pack ODS-AM column with an isocratic elution by solution A containing 30 mM sodium hydroxide at a flow rate of 0.8 ml/min.

Preparation of 8-OH-rGTP

8-OH-rGTP was prepared by oxidation of GTP (20 mg) in 16 ml of an aqueous solution of 50 mM sodium phosphate buffer, pH 7.0, 4 mg ascorbic acid and 0.4 % hydrogen peroxide at room temperature for 3 h in the dark (3). 8-OH-rGTP was purified by reverse-phase HPLC using a YMC-Pack ODS-AM column with isocratic elution by 0.5% acetic acid at a flow rate of 1 ml/min. The 8-OH-rGTP fraction was then purified by two YMC-Pack ODS-AM columns connected in series, with an isocratic elution by 0.5% acetic acid and 5 mM sodium dihydrogenphosphate at a flow rate of 0.6 ml/min. The 8-OH-rGTP fraction was further purified by a TSK-GEL DEAE-2SW column with an isocratic elution by 300 mM triethyl-ammonium hydrogencarbonate at a flow rate of 0.6 ml/min. The triethylammonium hydrogencarbonate was removed from the preparations as described above. The nucleoside obtained by dephosphorylation with bacterial alkaline phosphatase was identified as 8-hydroxy-Guo by its spectroscopic and chromato-graphic behaviors on reverse-phase HPLC, using a YMC-Pack ODS-AM column with isocratic elution by solution A containing 30 mM sodium hydroxide at a flow rate of 0.8 ml/min. 8-hydroxy-GMP and 8-hydroxy-GDP were prepared by a similar procedure.

Preparation of 8-OH-rATP

8-OH-rATP was prepared by a procedure similar to that reported for 8-hydroxy-AMP (25). Aqueous buffered (66 mM sodium phosphate, pH 6.0) solutions of 4 mM ATP were treated with 25 μM *meso*-tetrakis-(*N*-methyl-4-pyridyl)porphyrinato-manganese(III) (Aldrich) and 25 mM potassium monopersulfate at room temperature for 30 min. The reaction mixture was separated by reverse-phase HPLC using two YMC-Pack ODS-AM columns connected in series, with isocratic elution by solution A containing 30 mM sodium hydroxide at a flow rate of 0.6 ml/min. The 8-OH-rATP fraction was further purified by anion-exchange HPLC using a TSK-GEL DEAE-2SW column with an isocratic elution consisting of 300 mM triethylammonium hydrogen-carbonate at a flow rate of 0.6 ml/min. The triethylammonium hydrogencarbonate was removed from the preparations as described above. The nucleoside obtained by dephosphorylation with bacterial alkaline phosphatase was identified as 8-hydroxy-Ado by its spectroscopic and chromatographic behaviors on reverse-phase HPLC, using a YMC-Pack ODS-AM column with isocratic elution by solution A containing 30 mM sodium hydroxide at a flow rate of 0.8 ml/min.

Assay for MTH1 pyrophosphatase activity

The MTH1 activities were assayed in a reaction mixture (100 μl) containing 20 mM Tris–HCl, pH 8.0, 4 mM MgCl$_2$,
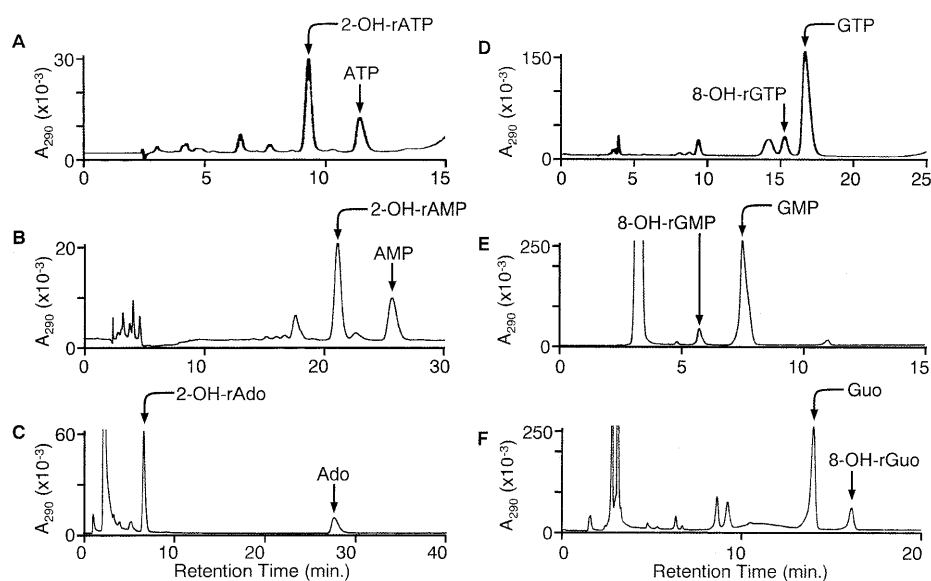
Figure 1. Formation of the oxidized nucleotides and nucleosides by ROSs. The nucleotides and nucleosides were treated with Fe(II)–EDTA and were analyzed by HPLC as described in the Materials and Methods. The production of (**A**) 2-OH-rATP from ATP, (**B**) 2-OH-rAMP from AMP, (**C**) 2-OH-rAdo from Ado, (**D**) 8-OH-rGTP from GTP, (**E**) 8-OH-rGMP from GMP and (**F**) 8-OH-rGuo from Guo are shown.

40 mM NaCl, 80 µg/ml bovine serum albumin, 8 mM dithio-threitol, 10% glycerol and various amounts of the nucleotide substrates. Following a preincubation at 30°C for 2 min, the mixtures were then incubated at 30°C for 5 min with the MTH1 protein. Reactions were terminated by adding 100 µl of ice-cold 5 mM EDTA. All samples were injected into a TSK-GEL DEAE-2SW column, with isocratic elution by 75 mM sodium phosphate buffer, pH 7.0, 20% acetonitrile and 1 mM EDTA at a flow rate of 1 ml/min. The nucleoside triphosphates and their hydrolyzed products were quantified by measuring the area of UV absorbance.

RESULTS

Formation of 2-OH-rATP by ROS

Aqueous solutions of 1 mM ATP or GTP were treated with Fe(II)–EDTA, an ROS-generating reagent, as described previously (14). This Fenton-type reagent has been used as a model of oxidation reactions in cells. The reaction mixtures were fractionated by HPLC, and the oxidized products were quantified by measuring the area of UV absorbance. As shown in Figure 1A and D, 2-OH-rATP and 8-OH-rGTP were produced upon ROS-treatment of ribonucleotides. The yields of 2-OH-rATP and 8-OH-rGTP were 1.4 and 2.2%, respectively (Table 1). The ratio of the yield of 2-OH-rATP to that of 8-OH-rGTP was 1:1.6. Thus, 2-OH-rATP is expected to be generated at a level similar to that of 8-OH-rGTP in cells. The amount of cellular ATP is 4.5-fold greater than that of GTP (21). Thus, the amount of 2-OH-rATP in the cell may be several-fold higher than that of 8-OH-rGTP. No peak corresponding to 8-OH-rATP was detected (Fig. 1A). The oxidation experiments of AMP, GMP, Ado and Guo gave similar results to those of ATP and GTP (Fig. 1, Table 1).

Table 1. Formation of the oxidized nucleotides and nucleosides by treatment with Fe(II)–EDTA

	ATP/GTP	AMP/GMP	Ado/Guo
2-OH-Ade	1.4	1.2	1.6
8-OH-Gua	2.2	1.8	3.8

Yields (%) were obtained by measuring the areas of the profiles in Figure 1.

Hydrolysis of oxidized ribonucleotides by MTH1 protein

The MTH1 activities for 8-OH-dGTP, 8-OH-rGTP, 8-OH-rATP and 2-OH-rATP were measured. each substrate was mixed with the MTH1 protein and was incubated at 30°C for 5 min. The hydrolyzed products were separated and quantified by anion-exchange HPLC. Under our conditions, the nucleoside triphosphates and their cognate di- and monophosphates were clearly separated (Fig. 2A). When these nucleotides were incubated with MTH1, the products (monophosphates) were formed in the order of 8-hydroxy-dGMP > 2-hydroxy-AMP >> 8-hydroxy-GMP ≈ 8-hydroxy-AMP (Fig. 2C–F). It was found that 46% of the 2-OH-rATP was hydrolyzed, under the conditions where 84% of the 8-OH-dGTP was hydrolyzed (Fig. 2E and F). In contrast, <6% of the 8-OH-rGTP and the 8-OH-rATP was hydrolyzed (Fig. 2C and D).

To compare the MTH1 pyrophosphatase activities for 2-OH-rATP, 8-OH-rGTP, 8-OH-rATP, ATP, GTP, 8-OH-dGTP and dGTP, 5 µM of these substrates were incubated with various amounts of the MTH1 protein. The 8-OH-rGTPase activity of MTH1 was 40-fold lower than the 8-OH-dGTPase activity at the 5 µM substrate concentration (Fig. 3A), and was 30-fold
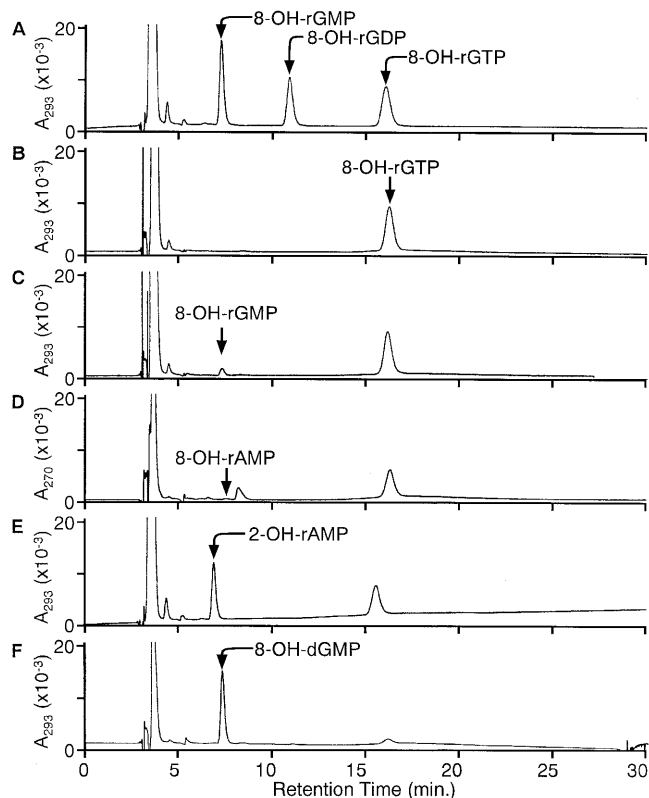
Figure 2. Hydrolysis of oxidized ribonucleotides by MTH1, monitored by anion-exchange HPLC. (**A**) Separation of standard nucleotides, 8-OH-rGTP, 8-OH-rGDP and 8-OH-rGMP. (**B** and **C**) 5 μM 8-OH-rGTP incubated with MTH1 at 30°C for 0 and 5 min, respectively. (**D**, **E** and **F**) 5 μM 8-OH-rATP, 2-OH-rATP and 8-OH-dGTP, respectively, incubated with MTH1 at 30°C for 5 min.

Figure 3. Specific hydrolysis of 2-OH-rATP by MTH1. (**A**) 5 μM of nucleotide substrates incubated with various amounts of MTH1 at 30°C for 5 min. (**B**) Expanded view of (A) at low concentrations of MTH1. Only the data for 8-OH-dGTP, 2-OH-rATP and dGTP are shown.

lower than the 8-OH-dGTPase activity at 20 μM (data not shown). These results were similar to those reported by Hayakawa *et al.* (11), who found a 50-fold difference in the hydrolysis rate of 8-OH-dGTP versus 8-OH-rGTP. On the other hand, the 2-OH-rATPase activity of MTH1 was much higher than the activities for 8-OH-rGTP and 8-OH-rATP, and was one-fifth of the rate of the 8-OH-dGTPase activity (Fig. 3B). The rates of the 8-OH-rGTPase and 8-OH-rATPase activities were lower than that of the dGTPase activity under these conditions (half and one-fifth, respectively; Fig. 3A). The rates of the GTPase and ATPase activities were the lowest among the substrates tested.

Kinetic parameters of MTH1 for oxidized ribonucleotides

To define the pyrophosphatase activities for three of the oxidized ribonucleotides, various amounts of 2-OH-rATP, 8-OH-rGTP and 8-OH-rATP were incubated with 1 nM MTH1 for 2-OH-rATP and 20 nM MTH1 for the other damaged ribonucleotides (Fig. 4). These MTH1 concentrations were optimized for the pyrophosphatase assay, based on the results in Figure 3. The Michaelis constant (K_m) and the catalytic constant (k_{cat}) of the reactions catalyzed by the MTH1 protein were calculated (Table 2).
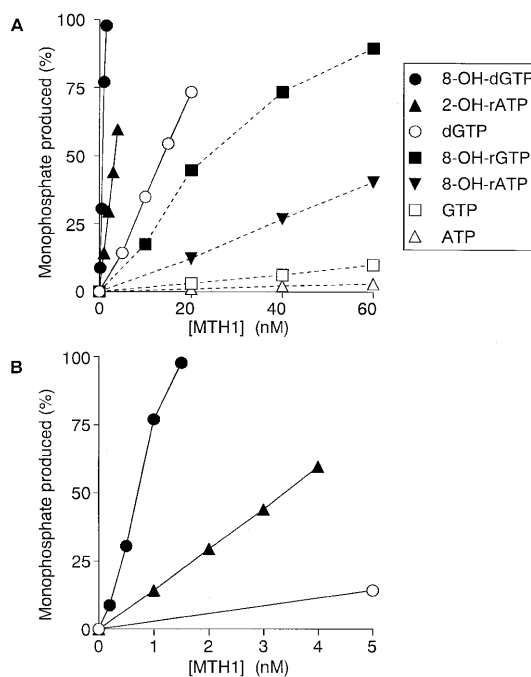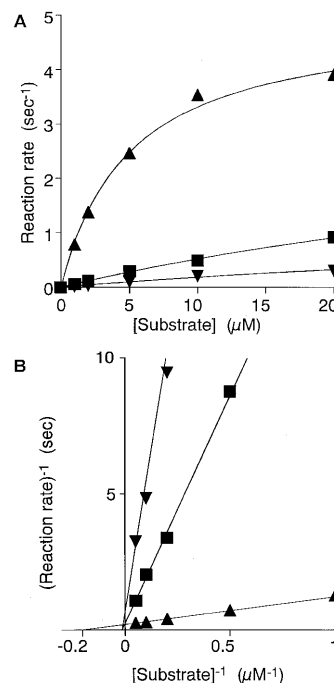

Figure 4. (**A**) Reaction rate versus substrate concentration plots and (**B**) Lineweaver–Burk plots for the MTH1 activities. The data for 2-OH-rATP (triangles), 8-OH-rGTP (squares) and 8-OH-rATP (inverted triangles) were obtained from the HPLC assay, as described in the Materials and Methods. Curves were fitted to the Michaelis–Menten equation based on the kinetic parameters calculated in Table 2.

Table 2. Substrate specificity of MTH1

Substrate	K_m (µM)	k_{cat} (s^{-1})[a]	k_{cat}/K_m (s^{-1}µM^{-1})
2-OH-rATP	4.3	4.7	1.09
8-OH-rGTP	55	2.6	0.05
8-OH-rATP	51	1.1	0.02
2-OH-dATP[b]	8.3	13.9	1.68
8-OH-dGTP[b]	15.2	12.3	0.81
8-OH-dATP[b]	13.9	10.8	0.78
dGTP[b]	258	15.7	0.06

The reaction mixtures, containing 1–20 µM of substrate were incubated with 1 nM MTH1 (for 2-OH-rATP) or 20 nM MTH1 (for 8-OH-rGTP and 8-OH-rATP) for 5 min at 30°C.
[a]The k_{cat} value was calculated as molecules of product formed per molecule of MTH1 per second.
[b]These values are from Fujikawa *et al.* (12).

Of the oxidized ribonucleotides tested, 2-OH-rATP was the best substrate for MTH1. 2-OH-rATP was hydrolyzed with the highest affinity and with the highest catalytic efficiency. Unexpectedly, the 2-OH-rATPase activity was slightly higher (k_{cat}/K_m = 1.09) than the 8-OH-dGTPase activity (k_{cat}/K_m = 0.81). The K_m value for 2-OH-rATP was 3.5-fold lower than that for 8-OH-dGTP, although the k_{cat} value was 2.6-fold lower. Thus, it was expected that the MTH1 protein would hydrolyze 2-OH-rATP more efficiently than 8-OH-dGTP when the substrate concentrations are low, as in the situation in living cells. In fact, the ratios of 2-OH-rATPase activity to 8-dGTPase activity were 110, 115 and 134% at substrate concentrations of 1, 0.5 and 0.1 µM, respectively (data not shown). These results agree with the theoretical values based on the kinetic parameters calculated in Table 2. Among the substrates, the strongest affinity (K_m = 4.3 µM) was found for 2-OH-rATP. The affinity for 2-OH-rATP was 1.9-fold stronger than that for 2-OH-dATP. Due to this high affinity, the k_{cat}/K_m value for 2-OH-rATP was only 1.5-fold less than that for 2-OH-dATP, the best substrate identified thus far.

The kinetic parameters for 8-OH-rGTP and 8-OH-rATP indicate that these ribonucleotides had ~3.5-fold lower affinity and 5–10-fold lower catalytic efficiency than the cognate deoxyribonucleotides. These properties made the 8-OH-rGTPase and 8-OH-rATPase activities of MTH1 very low. 8-OH-rGTP and 8-OH-rATP were hydrolyzed with 1/16th~1/40th efficiency as compared with 8-OH-dGTP and with either similar or lower efficiency as compared with undamaged dGTP.

The affinity for 2-OH-rATP was much higher than 8-OH-rGTP and 8-OH-rATP. The catalytic efficiencies of MTH1 were in the order of 2-OH-dATP > 2-OH-rATP > 8-OH-dGTP > 8-OH-dATP >> dGTP > 8-OH-rGTP > 8-OH-rATP. Thus, 2-OH-rATP appears to be removed from the ribonucleotide pool in human cells.

DISCUSSION

Bacterial MutT efficiently hydrolyzes 8-OH-rGTP as well as 8-OH-dGTP and suppresses transcription errors (8). On the other hand, the human MutT homologue, the MTH1 enzyme, has slight 8-OH-rGTP hydrolysis activity, although it hydrolyzes 8-OH-dGTP efficiently (11). We recently showed that MTH1 efficiently hydrolyzes three oxidized purine deoxyribonucleotides, 2-OH-dATP, 8-OH-dATP and 8-OH-dGTP (12). In the present study, we found that the human MTH1 protein specifically hydrolyzed 2-OH-rATP. 2-OH-rATP was hydrolyzed with very high efficiency and, unexpectedly, with the highest affinity among the known substrates. Since the difference in the catalytic efficiencies between the 8-OH-dGTPase and dGTPase activities of MTH1 is only ~10–70-fold, in contrast to that of MutT (2000-fold), the physiological significance of the 8-OH-dGTPase activity of MTH1 has remained unclear (4,10,this paper). The very low activities of MTH1 for ATP and GTP suggest that MTH1 clearly distinguishes between 2-OH-rATP and these undamaged ribonucleotides (Fig. 3). The main reason why the K_m values of MTH1 for 2-OH-rATP and dGTP were relatively close (Table 2) may be due to the fact that the ATP pool size is >100 times larger than the dGTP pool size (21). These results suggest that 2-OH-rATP is an intrinsic substrate for the human MTH1 enzyme. However, the MTH1 efficiencies for 8-OH-rGTP and 8-OH-rATP were similar or lower as compared with that for undamaged dGTP. These results indicate that the MTH1 enzyme has no function in the elimination of 8-OH-rGTP and 8-OH-rATP from the RNA precursor pool. Taken together, the human MTH1 enzyme hydrolyzes 2-OH-rATP, 2-OH-dATP, 8-OH-dGTP and 8-OH-dATP among the oxidized nucleotides tested. These findings provide the possibility that the other enzyme may contribute to 8-OH-rGTP elimination from the RNA precursor pool in mammalian cells, because 8-OH-rGTP is degraded by the bacterial MutT protein as efficiently as 8-OH-dGTP (8).

The fact that 2-OH-rATP and 2-OH-dATP are the best substrates among the damaged ribo- and deoxyribonucleotides tested suggests that the human MTH1 protein recognizes 2-hydroxy-Ade nucleotides. As discussed in our previous paper (12), the MTH1 protein may interact with the functional group(s) of 2-hydroxy-Ade nucleotides. 8-OH-rGTP and 8-OH-rATP probably adopt the *syn*-conformation, because the cognate ribonucleosides shift toward the *syn*-conformation (26). Thus, our previous speculation that MTH1 may recognize the specific (*syn*) conformation is not applicable, at least in the case of ribonucleotides.

The catalytic constants (k_{cat}) for the three oxidized ribonucleotides were lower than the corresponding oxidized deoxyribonucleotides (and, in the case of 8-OH-rGTP, also lower than the k_{cat} for dGTP), irrespective of the substrate affinities. It is possible that the target phosphorus atoms of the ribonucleotides may be positioned differently to those of the cognate deoxyribonucleotides, due to differences in the configuration of the sugar moieties, or may be hard to dissociate from the recognition site.

ATP is an important material involved in RNA synthesis, energy metabolism and signal transduction. Many ATP analogs are known to inhibit these pathways (17–20). We have shown that 2-OH-rATP is efficiently produced by ROS-treatment of ATP. Since the amount of ATP is highest among the ribonucleoside triphosphates in human cells (21), it is likely that 2-OH-rATP is produced from ATP several-fold more frequently than 8-OH-rGTP from GTP in cells. Therefore, one of the MTH1 functions would be the elimination of 2-OH-rATP, which is produced efficiently in the ribonucleotide pool. It is possible that the

oxidized ATP induces transcriptional errors, like 8-OH-rGTP (8), because 2-OH-dATP is highly mutagenic (5). In addition, it is possible that 2-OH-rATP disturbs energy metabolism and signal transduction mediated by ATP. Further studies will be necessary to reveal the effects of 2-OH-rATP in transcription and ATP-related processes.

ACKNOWLEDGEMENTS

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