

# 8-Chloro-dGTP, a hypochlorous acid-modified nucleotide, is hydrolyzed by hMTH1, the human MutT homolog

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**Abstract** The human mutT homolog, hMTH1, suppresses spontaneous mutations by degrading the endogenous mutagen, 8-hydroxy-dGTP. We previously reported the broad substrate specificity of hMTH1, which also degrades the oxidatively damaged purine nucleotides, 2-hydroxy-dATP, 8-hydroxy-dATP, 2-hydroxy-ATP, and 8-hydroxy-GTP, in addition to 8-hydroxy-dGTP. In this paper, we describe the hMTH1 activity for 8-chloro-dGTP, which could be formed in inflamed tissue by the reaction of dGTP with hypochlorous acid, a product of myeloperoxidase from activated human neutrophils. The hMTH1 protein was mixed with 1–20  $\mu$ M of 8-chloro-dGTP and 8-hydroxy-dGTP, and the reaction products were quantified by anion-exchange HPLC to measure the pyrophosphatase reaction rate. The kinetic parameters revealed that 8-chloro-dGTP was degraded by hMTH1 with 50% efficiency as compared with that of 8-hydroxy-dGTP. This result suggests that 8-chloro-dGTP is an intrinsic substrate for hMTH1. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

**Key words:** 8-Chloro-dGTP; 8-Hydroxy-dGTP; hMTH1; mutT; Nucleotide sanitization enzyme

## 1. Introduction

Chronic infection by bacteria, parasites or viruses and tissue inflammation, such as gastritis and colitis, are recognized risk factors for human cancers at various sites [1]. Infection and inflammation activate a variety of inflammatory cells, which induce and activate various oxidant-generating enzymes. Activated human neutrophils secrete myeloperoxidase (MPO), which generates hypochlorous acid (HOCl) using hydrogen peroxide ( $H_2O_2$ ) and chloride ion ( $Cl^-$ ) as substrates. It has recently been reported that various chlorinated nucleosides, including the novel 8-chloro-2'-deoxyguanosine (8-Cl-dGuo), 5-chloro-2'-deoxycytidine, and 8-chloro-2'-deoxyadenosine, are formed by reactions of nucleosides with HOCl as well as with human MPO or activated human neutrophils in the presence of  $H_2O_2$  and  $Cl^-$  [2–4]. Among the naturally occurring nucleosides, 2'-deoxyguanosine reacts with HOCl or human

MPO to generate 8-Cl-dGuo with a high yield under physiological conditions, particularly in the presence of tertiary amines such as nicotine and trimethylamine, which act as catalysts to enhance the chlorination of nucleosides [2]. Although 8-Cl-dGuo has not been studied for mutagenicity, an analogous compound, 5-bromo-2'-deoxycytidine, is incorporated into DNA [5]. In addition, the incorporation of both dAMP and dCMP by human DNA polymerases occurred opposite the 8-bromo-2'-deoxyguanosine present in DNA templates, and induced G:C to T:A transversion mutations [6]. Thus, 8-Cl-dGuo is likely to be mutagenic, if it is incorporated or formed within DNA.

In the present study, as a prelude to an evaluation of the biological significance of this modified base, we have tested whether 8-Cl-dGTP, a 5'-triphosphate derivative of 8-Cl-Guo, is a substrate for the human enzyme hMTH1. This enzyme hydrolyzes various oxidized nucleotides, such as 8-hydroxy-dGTP and 2-hydroxy-dATP, to the corresponding nucleotide monophosphates, and acts as a nucleotide sanitization enzyme to prevent the incorporation of these modified nucleotides into DNA [7,8].

## 2. Materials and methods

### 2.1. Materials

8-OH-dGTP was prepared by the oxidation of dGTP (Sigma), and was purified by reverse-phase HPLC as described [8].

### 2.2. Production and purification of 8-Cl-dGTP

dGTP (5 mM; 400  $\mu$ l) was reacted with 5 mM NaOCl in 250 mM sodium phosphate buffer (pH 7.4) containing 125  $\mu$ M nicotine, a catalyst of chlorination caused by HOCl, at room temperature for 2 min. The reaction was stopped by the addition of 10  $\mu$ l of 500 mM *N*-acetylcysteine. The 8-Cl-dGTP in the reaction mixture was isolated by preparative reverse phase HPLC using the following sequential separation steps. At first, the 8-Cl-dGTP was isolated using an eluent of 20 mM sodium phosphate buffer (pH 7.0) containing 0.6% acetonitrile (isocratic). Then, the 8-Cl-dGTP was purified from the above HPLC fraction using an eluent of 50 mM triethylammonium acetate buffer (pH 7.0) containing acetonitrile. The acetonitrile concentration was increased from 0 to 10% for 20 min in a linear gradient mode. Further purification was done using an eluent of 20 mM triethylammonium bicarbonate buffer (pH 7.0) containing 4% acetonitrile (isocratic). Finally, the isolated 8-Cl-dGTP was repeatedly lyophilized after the addition of  $H_2O$  (three times) to remove the buffer. The purity of the 8-Cl-dGTP was evaluated by its UV spectrum, and by the profile from the reverse phase HPLC using 50 mM triethylammonium acetate buffer (pH 7.0) with a linear gradient of

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0–10% acetonitrile as the eluent. Detection was performed with a Hewlett Packard 1050 HPLC detection system. For the reverse phase HPLC, an Ultrasphere ODS column (4.6×250 mm, 5  $\mu$ m particle size, Beckman, CA, USA) was used. The column temperature was 30°C, and the flow rate was 1.0 ml/min. The concentration of 8-Cl-dGTP was determined by the UV absorbance and the molar extinction coefficient ( $\epsilon_{\text{max}} = 1.53 \times 10^4$ ,  $\lambda_{\text{max}} = 261$  nm at pH 7.0).

### 2.3. Pyrophosphatase assay

The pyrophosphatase activities were assayed in a reaction mixture (100  $\mu$ l) containing 20 mM Tris-HCl (pH 8.0), 4 mM  $\text{MgCl}_2$ , 40 mM NaCl, 80  $\mu$ g/ml bovine serum albumin, 8 mM dithiothreitol, 10% glycerol, and various amounts of nucleotide substrates. Following a preincubation at 30°C for 2 min, the mixtures were incubated at 30°C for 10 min with various amounts of the hMTH1 protein. Reactions were terminated by adding 100  $\mu$ l of 5 mM EDTA. All samples were injected into a TSK-GEL DEAE-2SW column, with an isocratic elution by 50 mM phosphate buffer (pH 7.0), 1 mM EDTA, and 20% acetonitrile at a flow rate of 1 ml/min. Detection was performed with a Hewlett Packard 1040M HPLC UV detection system. The nucleoside triphosphates and their hydrolyzed products were quantified by measuring the peak areas in the HPLC data.

## 3. Results and discussion

When the reaction mixtures were fractionated by anion-exchange HPLC, 8-Cl-dGTP and its hydrolyzed product, 8-Cl-dGMP, were clearly separated (Fig. 1A,B). With the standard conditions described in the figure legend, 8-Cl-dGTP was hydrolyzed to 8-Cl-dGMP with 70% efficiency as compared to the 8-OH-dGTP hydrolysis, and very little dGTP degradation was found with these conditions (Fig. 1B–D). The hydrolysis rates of the three nucleotides, 8-OH-dGTP, 8-Cl-dGTP, and dGTP, with various concentrations of hMTH1, are shown in Fig. 2. The 8-Cl-dGTP was hydrolyzed at 60–80% of the rate of 8-OH-dGTP. The hydrolyzing activity of dGTP was 1.5% as compared with that of 8-OH-dGTP. Next, various amounts (1–20  $\mu$ M) of 8-Cl-dGTP and 8-OH-dGTP were mixed with 1 nM hMTH1 protein, and the reaction rates were measured by anion-exchange HPLC to determine the kinetic parameters

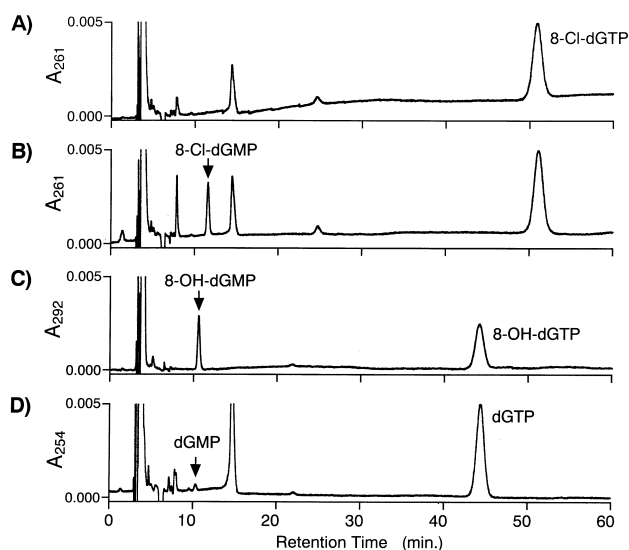


Fig. 1. Hydrolysis of oxidized nucleotides by hMTH1, monitored by anion-exchange HPLC. A, B: 5  $\mu$ M 8-Cl-dGTP incubated with hMTH1 at 30°C for 0 and 5 min, respectively. C, D: 5  $\mu$ M 8-OH-dGTP and dGTP, respectively, incubated with hMTH1 at 30°C for 5 min.

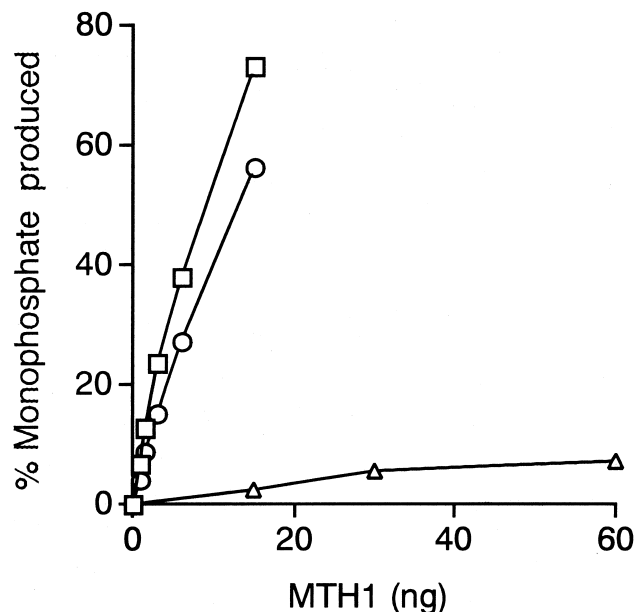


Fig. 2. Hydrolysis of 8-Cl-dGTP by hMTH1. Nucleotide substrates (5  $\mu$ M) were incubated with various amounts of hMTH1 at 30°C for 5 min. The data for 8-Cl-dGTP (circles), 8-OH-dGTP (squares), and dGTP (triangles) are shown.

(Fig. 3). The kinetic efficiency ( $k_{\text{cat}}/K_m$ ) of hMTH1 for 8-Cl-dGTP was half of that for 8-OH-dGTP (Table 1).

8-OH-dGDP is a strong, and competitive inhibitor of the hMTH1 activity [7]. The inhibition constants ( $K_i$ ) of 8-OH-dGDP are 0.72  $\mu$ M against the 2-OH-dATPase activity, and 0.51  $\mu$ M against the 8-OH-dGTPase activity. The inhibitory effect of 8-OH-dGDP against the 8-Cl-dGTP activity of hMTH1 was studied. Various amounts of 8-OH-dGDP (0–0.5  $\mu$ M) were added to the reaction mixtures containing 1–10  $\mu$ M of 8-Cl-dGTP, and were incubated with 1 nM of hMTH1. The inhibition constant ( $K_i$ ) of 8-OH-dGDP against the 8-Cl-dGTPase activity of hMTH1 was 0.43  $\mu$ M. In addition, 8-OH-dGTP (5  $\mu$ M) and 8-Cl-dGTP (5  $\mu$ M) were mixed, and then incubated with 2 nM of hMTH1. The 8-OH-dGTPase activity and the 8-Cl-dGTPase activity were reduced to 92.4% and 76.3%, respectively, as compared with the activities for each damaged nucleotide alone. These results are in agreement with our finding that 8-Cl-dGTP is recognized with lower affinity than 8-OH-dGTP, and indicate that the damaged bases of these nucleotides share the same recognition site of hMTH1.

We have previously reported that the oxidized purine nucleotides, 2-OH-dATP, 8-OH-dATP, and 8-OH-dGTP, and 2-OH-ATP, which have the *syn*-conformation, are all good substrates for hMTH1 [7,9]. Therefore, our present result that 8-Cl-dGTP, which may adopt the *syn*-conformation

Table 1  
Substrate specificity of hMTH1

Substrate	$K_m$ ( $\mu$ M)	$k_{\text{cat}}$ ( $\text{s}^{-1}$ )	$k_{\text{cat}}/K_m$ ( $\text{s}^{-1} \mu\text{M}^{-1}$ )
8-Cl-dGTP	58.5	19.0	0.33
8-OH-dGTP	18.9	12.4	0.66

The reaction mixtures, containing various substrates (1–20  $\mu$ M of 8-Cl-dGTP or 8-OH-dGTP), were incubated with 1 nM hMTH1 for 5 min at 30°C. The  $k_{\text{cat}}$  value was calculated as molecules of product formed/molecule of hMTH1/s.

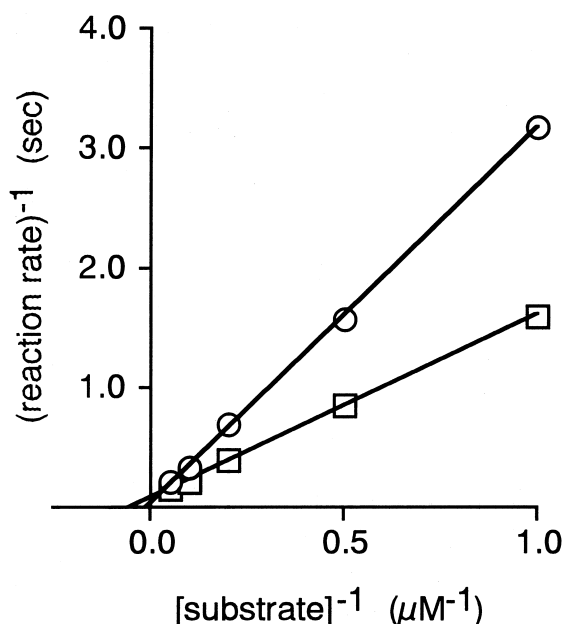


Fig. 3. Lineweaver–Burk plots for the hMTH1 activities. The data for 8-Cl-dGTP (circles) and 8-OH-dGTP (squares) were obtained from the HPLC assay, as described in Section 2. Curves were fitted to the Michaelis–Menten equation.

[10], is efficiently hydrolyzed by hMTH1 is compatible with this finding. Bessman et al. have reported that 8-Br-dATP and 8-Br-dGTP are hydrolyzed by the *Escherichia coli* Mut T protein at a higher rate than unmodified dATP and dGTP [11]. Our preliminary experiments showed that 8-Cl-dGTP may be a poor substrate for *E. coli* MutT (data not shown). This is reasonable, because MPO-dependent hypochloric acid formation is specific to the mammalian system and is not expected to occur in bacterial systems.

Our present result that 8-Cl-dGTP is a good substrate of the hMTH1 protein suggests that 8-Cl-dGTP may be produced in human cells. The detection of 8-Cl-Gua in cellular DNA and in the nucleotide pool and its mutagenic effects and repair mechanisms are important projects to be pursued in the future.

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