

Differential inhibition of eukaryotic DNA polymerases by halenaquinol sulfate, a *p*-hydroquinone sulfate obtained from a marine sponge

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Abstract

Halenaquinol sulfate, a *p*-hydroquinone sulfate obtained from a marine sponge, inhibited the activity of eukaryotic DNA polymerases in varying degrees; the K_i values for DNA polymerases α , β , δ and ϵ were 1.3, 80, 17.5 and 2.0 μ M, respectively, whereas it was less effective against *E. coli* DNA polymerase I. The inhibition occurred competitively with each of dATP and dTTP, but non-competitively with dCTP, dGTP and the template DNA. Thus, halenaquinol sulfate is demonstrated to be a potential inhibitor of DNA polymerases α and ϵ , and be a useful tool for analyzing the dNTP binding sites of DNA polymerases.

Key words: Halenaquinol sulfate; DNA polymerase α ; DNA polymerase ϵ ; DNA polymerase δ ; Inhibition

1. Introduction

Recent investigations have revealed that eukaryotic cells contain at least five types (α , β , γ , δ , and ϵ) of DNA polymerase [1–3]. DNA polymerases α and δ are required for nuclear DNA replication, whereas DNA polymerase γ participates in mitochondrial DNA replication [1–2]. DNA polymerases β and ϵ have been implicated to be involved in DNA repair, but there are also reports suggesting that these enzymes are related to recombination and DNA replication, respectively [1–3]. However, all function of eukaryotic DNA polymerases has not fully been elucidated. Previously, we found that aphidicolin is a selective inhibitor of both DNA polymerase α and eukaryotic DNA replication, thereby revealing that this polymerase is essential for DNA replication [4]. Thus, selective inhibitors of DNA polymerases are useful tools to distinguish DNA polymerases and to clarify their biological function [1]. However, there are very few inhibitors capable of distinguishing between DNA polymerases α , δ and ϵ . In the present study, we show that halenaquinol sulfate (Fig. 1), which is obtained from the sponge *Xestospongia sapra* and which is known to be an inhibitor of protein tyrosine kinase [5] as well as an inhibitor of cell membrane fusion [6], is a novel, selective inhibitor of DNA polymerases α and ϵ with the competitive mode of inhibition with respect to dATP and dTTP.

2. Materials and methods

2.1. Inhibitors

Halenaquinol sulfate was purified from the sponge *Xestospongia sapra*, as described [7,8]. This compound was dissolved into ethanol and was directly diluted in the reaction mixture. Aphidicolin and heparin were obtained from Wako Pure Chemicals (Osaka, Japan) and Boehringer Mannheim (Mannheim, Germany), respectively.

2.2. DNA polymerases

DNA polymerase α was purified from calf thymus [9], human Raji cells [9] and *Xenopus* ovary [10], as described previously. DNA polymerase β was prepared from calf thymus [11] and rat ovary [12], as described. DNA polymerase γ was purified from human acute myelogenous leukemia cells, as described [13]. DNA polymerases δ and ϵ were purified from an extract of calf thymus using column chromatographies with DEAE-cellulose, hydroxylapatite, Mono Q and Mono S, according to the procedure described by Weiser et al. [14] with modifications (this procedure will be described in more detail elsewhere). *E. coli* DNA polymerase I and avian myeloblastosis virus reverse transcriptase were obtained from Takara (Kyoto, Japan).

2.3. Assay for DNA polymerases

Each DNA polymerase (0.01–0.05 units) was incubated with the indicated concentration of inhibitors in a standard reaction mixture at 37°C for 30 min. The standard reaction mixture consisted of 25 mM Tris-HCl (pH 7.5), 50 mM KCl, 7 mM MgCl₂, 10 μ M each of dATP, dGTP and dTTP, 2.5 μ M dCTP containing 1.0 μ Ci [³H]dCTP, 0.1 mg/ml activated calf thymus DNA, 0.4 mg/ml bovine serum albumin and 10% glycerol in a final volume of 25 μ l. For DNA polymerase γ and reverse transcriptase, the activated DNA and [³H]dCTP were replaced by poly(rA)-(dT)₁₀ (40 μ g/ml) and [³H]dTTP, respectively. After incubation, DNA polymerase activity was determined by the incorporation of radioactive deoxyribonucleoside 5'-monophosphates (dNMPs) into the acid-insoluble material, as described [9].

3. Results and discussion

The inhibitory effect of halenaquinol sulfate on various kinds of DNA polymerase is summarized in Table 1. Dose dependency of the inhibition is shown in Fig. 2.

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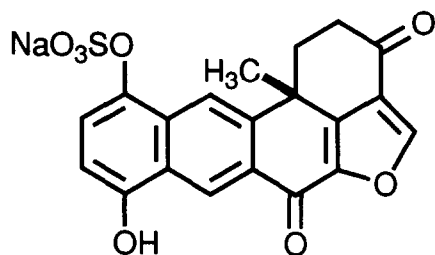


Fig. 1. Structure of halenaquinol sulfate.

In the concentration range of up to 200 μM of halenaquinol sulfate, the activity of all eukaryotic DNA polymerases was suppressed to a certain extent, whereas only 5% and 6% inhibition of the activity of *E. coli* DNA polymerase I and avian reverse transcriptase, respectively, resulted with the concentrations of 200 μM and 20 μM , respectively (Fig. 2 and Table 1). Thus it is unlikely that halenaquinol sulfate acts simply as a chelator of divalent cations or a destructor of substrates, but this sensitivity reflects the characteristic property of each eukaryotic DNA polymerase. To obtain a half inhibition, 5 and 7 μM concentrations of halenaquinol sulfate were required for DNA polymerases α and ϵ , respectively, while 100, 50 and 40 μM concentrations of halenaquinol sulfate were needed for DNA polymerases β , γ and δ , respectively (Fig. 2). Twenty micromolar concentration of halenaquinol sulfate inhibited almost all of the DNA polymerase α and ϵ activities, while the activity of polymerases β , γ and δ decreased to 92%, 85% and 75%, respectively, of the original level (Table 1). The sensitivity of DNA polymerases α and β to the inhibitor was unaltered regardless of animal or tissue species from which DNA polymerases were prepared (Table 1). Under the same assay conditions, 30 μM aphidicolin inhibited DNA polymerases α , δ and ϵ significantly, but not DNA polymerases β and γ (Table 1), in accordance

Table 1
The inhibitory effects of halenaquinol sulfate, heparin and aphidicolin on various kinds of DNA polymerase

DNA polymerase (Source)	Inhibition of activity (%)		
	Halenaquinol sulfate (20 μM)	Heparin (0.4 $\mu\text{g/ml}$)	Aphidicolin (30 μM)
α (human Raji cell)	92	95	94
α (calf thymus)	95	96	95
α (<i>Xenopus</i> ovary)	93	94	85
β (calf thymus)	8	2	4
β (rat ovary)	5	3	4
γ (human leukemia cell)	15	—	—
δ (calf thymus)	26	96	94
ϵ (calf thymus)	90	93	94
I (<i>E. coli</i>)	2	5	4
Reverse transcriptase (avian myeloblastosis virus)	6	—	—

with the previous results [15]. Remarkably, DNA polymerase δ was highly sensitive to aphidicolin, but not to halenaquinol sulfate (Table 1).

As shown in Fig. 1, halenaquinol sulfate is a hydroquinone containing an O-sulfate group. Compounds possessing sulfate groups would be expected to inhibit some DNA polymerase activity, although other structural features are important in producing inhibition [16,17]. Thus, we compared the inhibitory effect of halenaquinol sulfate on DNA polymerases with that of heparin, a polysaccharide containing sulfate groups, which is known to inhibit some eukaryotic DNA polymerases significantly [17,18]. Heparin (0.4 $\mu\text{g/ml}$) inhibited almost all of the activity of DNA polymerases α , δ and ϵ , but not of that of DNA polymerases β and I (Table 1), these results being consistent with previous ones [17,18]. Thus, the sensitivity of DNA polymerases α , β and ϵ to heparin was similar to that to halenaquinol sulfate. On the other hand, DNA polymerase δ is sensitive to heparin, but relatively resistant to halenaquinol sulfate (Table 1). This suggests that the mode of inhibition of DNA polymerases produced by halenaquinol sulfate differs from that of heparin.

To elucidate the mode of inhibitory action of halenaquinol sulfate on DNA polymerase α , we examined the relationship between the concentration of each deoxyribonucleoside 5'-triphosphate (dNTP) and the efficiency of inhibition (Fig. 3). Lineweaver–Burk plots of the relationship clearly reveal that by varying the concentration of either one of dATP and dTTP, the apparent K_m value increased with an increase of the halenaquinol sulfate concentration, whereas the apparent V_{max} remained unchanged (Fig. 3). On the other hand, by

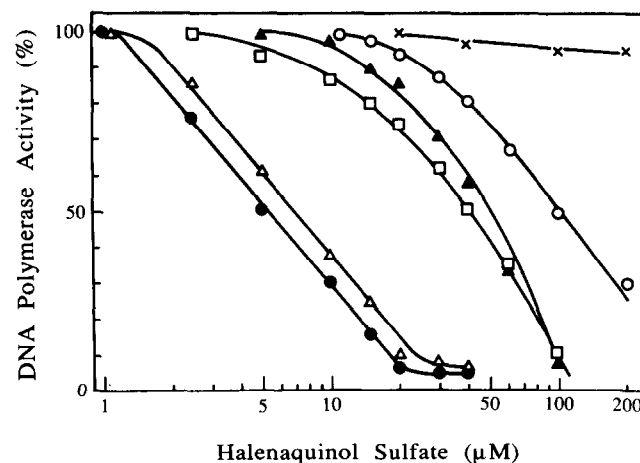


Fig. 2. Dose dependency of the inhibitory effect of halenaquinol sulfate on eukaryotic DNA polymerase activity. DNA polymerase activity was assayed in the presence of the indicated concentration of halenaquinol sulfate, as described in the text. ●, DNA polymerase α (calf thymus); ○, DNA polymerase β (calf thymus); ▲, DNA polymerase γ (human); △, DNA polymerase δ (calf thymus); □, DNA polymerase ϵ (calf thymus); X, DNA polymerase I (*E. coli*).

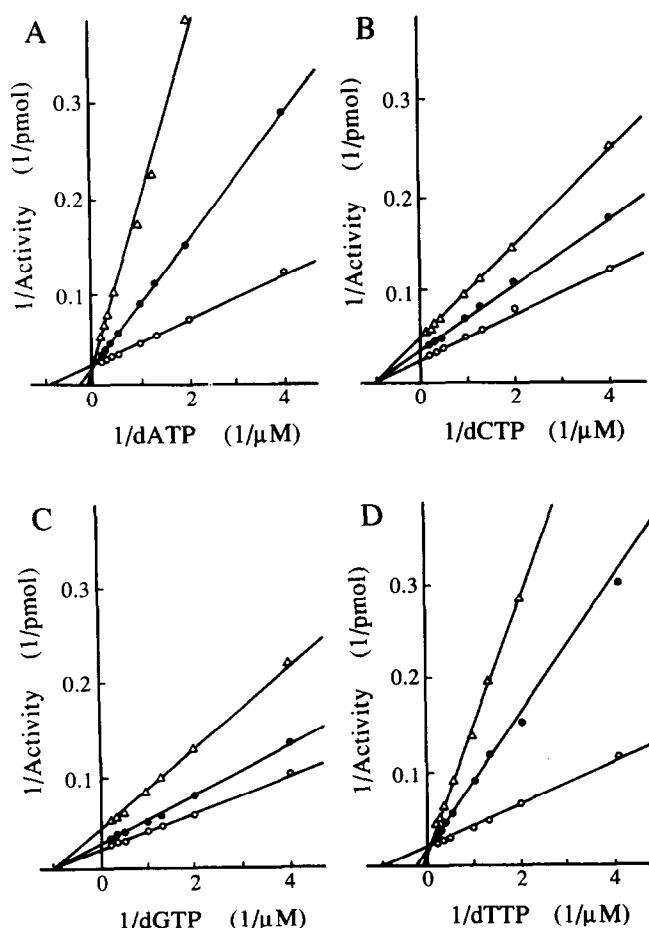


Fig. 3. Lineweaver-Burk plots showing the effects of the concentration of deoxyribonucleotides on inhibition of DNA polymerase α by halenaquinol sulfate. DNA polymerase α (calf thymus) activity, expressed as pmol dNMP incorporated during 30 min, was determined in the presence of halenaquinol sulfate ($\circ$, 0 μ M; $\bullet$, 4 μ M and $\triangle$, 6 μ M) as described in the text, except that the concentration of one (A, dATP; B, dCTP; C, dGTP and D, dTTP) of four dNTPs was varied in the presence of 20 μ M each of the other three dNTPs. The results were shown by Lineweaver-Burk plots. The apparent K_m values in the presence of 0, 4 and 6 μ M halenaquinol sulfate are 1.1, 3.6 and 10 μ M, respectively, for dATP; 1.0, 1.0 and 1.1 μ M, respectively, for dCTP; 0.98, 1.0 and 1.0 μ M, respectively, for dGTP; and 1.1, 3.8 and 9.0 μ M, respectively, for dTTP.

varying the concentration of dCTP or dGTP, the V_{max} decreased, but the K_m remained unchanged, with an increase of the halenaquinol sulfate concentration (Fig. 3). Next, the relationship between the amount of activated calf thymus DNA (template DNA) and the inhibition of the enzymatic activity was kinetically examined. It was found that the V_{max} decreased, but the K_m remained unchanged, with an increase of the template DNA concentration (data not shown). From these results, it is evident that halenaquinol sulfate inhibits DNA polymerase α competitively with only dATP and dTTP, but non-competitively with dCTP, dGTP and the template DNA. On the other hand, heparin inhibited DNA polymerase α

Table 2

The K_i values of halenaquinol sulfate for DNA polymerases

DNA polymerase (calf thymus)	K_m dATP (μ M)	K_i Halenaquinol sulfate (μ M)	K_m dTTP (μ M)	K_i Halenaquinol sulfate (μ M)
α	1.1	1.2	1.0	1.3
β	6.7	64.5	11.5	80.0
δ	1.4	16.2	1.1	17.5
ϵ	0.9	1.6	1.3	2.0

The K_i values of halenaquinol sulfate for DNA polymerases were determined with respect to each of dATP and dTTP, as described in the legend to Fig. 3.

non-competitively with dATP, dCTP, dGTP and dTTP, but competitively with the template DNA (data not shown), the results being essentially consistent with the previous ones [17]. This indicates that the nature of the inhibition produced by halenaquinol sulfate differs from that of heparin with respect to the mode of inhibitory action. At present, it remains unclear why halenaquinol sulfate, which is not a dNTP analogue, competes with dATP and dTTP. In this regard, it is notable that aphidicolin, which is not a derivative of dNTPs as well, is a competitive inhibitor of DNA polymerase α with respect to dCTP [19,20]. In contrast to halenaquinol sulfate, aphidicolin does not compete with dATP and dTTP [20].

The same mode of inhibitory action of halenaquinol sulfate as that for DNA polymerase α was observed for DNA polymerases β , δ and ϵ (data not shown). The K_i values of halenaquinol sulfate for these DNA polymerases (Table 2) show that halenaquinol sulfate inhibits eukaryotic DNA polymerases differentially and it is a potent inhibitor of DNA polymerases α and ϵ .

DNA polymerases α , β and γ have been distinguished by selective inhibitors such as aphidicolin, dideoxyTTP and *N*-ethylmaleimide [15]. It has been reported that butylphenyl-dGTP and buthylamilino-dATP inhibit DNA polymerases α efficiently, but δ and ϵ less [21–23]. On the other hand, carbonyldiphosphonate inhibits DNA polymerases δ efficiently, but α and ϵ less [23,24]. Judging from the sensitivity and the mode of inhibitory action, halenaquinol sulfate is a novel type of inhibitor of eukaryotic DNA polymerases. The use of halenaquinol sulfate will offer an opportunity to distinguish between DNA polymerase ϵ and the other eukaryotic DNA polymerases. The results described in this paper suggest that the dATP and dTTP binding sites in DNA polymerases α , β , δ and ϵ are distinct from the binding sites for the other two dNTPs. Therefore, halenaquinol sulfate is useful for analyzing the dNTP binding domain in each of DNA polymerase molecules.

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