The specific inhibitory effect of demethoxydehydroaltenusin, a derivative of dehydroaltenusin, on mammalian DNA polymerase α

ISOKO KURIYAMA1, KEISHI FUKUDOME2, SHINJI KAMISUKI2, KOUJI KURAMOCHI3, KAZUNORI TSUBAKI3, KENGO SAKAGUCHI2, FUMIO SUGAWARA2, HIROMI YOSHIDA1,4 and YOSHIYUKI MIZUSHINA1,4

1Laboratory of Food & Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180; 2Department of Applied Biological Science, Tokyo University of Science, Noda, Chiba 278-8510; 3Graduate School of Life and Environmental Science, Kyoto Prefectural University, Kyoto 606-8522; 4Cooperative Research Center of Life Sciences, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan

Received July 30, 2008; Accepted September 5, 2008

DOI: 10.3892/ijmm_00000087

Abstract. In the screening of selective inhibitors of eukaryotic DNA polymerases (pols), dehydroaltenusin from the fungus Acremonium sp. was found to be an inhibitor of pol α. The present study succeeded in chemically synthesizing dehydroaltenusin, and the compound strongly inhibited calf pol α activity and weakly suppressed rat pol β activity, with IC50 values of 0.68 and 64 μM, respectively. We purified or synthesized various slightly modified derivatives of dehydroaltenusin, and using these, we investigated the relationship between chemical structure and the inhibitory effects. These results suggest that the ketone group at the 5′-position in dehydroaltenusin is essential for pol inhibitory activity, and the group at the 5′-position is important for the specificity of pol α inhibition. Demethoxydehydroaltenusin was found to be the most specific pol α inhibitor among the prepared derivatives, and the IC50 values for pols α and β were 0.24 and 89 μM, respectively. This compound did not influence the activities of other replicative pols such as pols δ and ε, and also demonstrated no effect on pol α activity from another vertebrate, fish and a plant species. Demethoxydehydroaltenusin also had no influence on the other pols and DNA metabolic enzymes tested. Therefore, demethoxydehydroaltenusin is of interest as a mammalian pol α-selective inhibitor as a 'chemical knockout agent' in vitro and in vivo.

Introduction

The human genome encodes 16 DNA polymerases (pols) that control cellular DNA synthesis (1). Eukaryotic cells reportedly contain three replicative types: pols α, δ and ε, mitochondrial pol γ, and at least twelve repair types: pols β, δ, ε, ζ, η, θ, τ, κ, λ, μ, and ε and REV1 (2). Selective inhibitors of each pol are useful tools and molecular probes for distinguishing pols and for clarifying their biological and in vivo functions (3). For example, aphidicolin is a selective inhibitor of both pol α and eukaryotic DNA replicative pols δ and ε, indicating that these pols are essential for DNA replication (4), and this inhibitor has been very useful for studying the DNA replication system (5); however, aphidicolin is not capable of distinguishing pols α, δ and ε.

Therefore, we established an assay method to detect pol inhibitors, and have screened for natural sources of inhibitors for more than 10 years. An inhibitor was isolated that selectively influences the activity of mammalian pol α, dehydroaltenusin, from a fungus (Acremonium sp.) collected from fields in the vicinity of Noda city in Chiba prefecture, Japan (6). A total chemical synthesis method was established for dehydroaltenusin and succeeded in completely synthesizing the compound (7-9).

Subsequently, the slightly modified derivatives were purified or chemically synthesized to examine the structural relationship between dehydroaltenusin and pols. Dehydroaltenusin and its analogues represent a group of potentially useful agents to examine the precise role of each pol in vivo, and to develop a drug design strategy for cancer chemotherapy agents. The compounds can also be useful tools as molecular probes to study the three-dimensional structure of mammalian pol α protein.

Materials and methods

Materials. Nucleotides and chemically synthesized DNA template-primers such as [3H]-2′-deoxythymidine 5′-tri-

Correspondence to: Dr Yoshiyuki Mizushima, Laboratory of Food & Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan E-mail: mizushin@nutr.kobegakuin.ac.jp

Abbreviations: pol, DNA-directed DNA polymerase (EC 2.7.7.7); IC50, 50% inhibitory concentration; dTTP, 2′-deoxythymidine 5′-triphosphate; dNTP, 2′-deoxyribonucleotide 5′-triphosphate; HIV-1, human immunodeficiency virus type-1

Key words: demethoxydehydroaltenusin, dehydroaltenusin, DNA polymerase α, enzyme inhibitor, chemical knockout agent, DNA replication
phosphate (dTTP, 43 Ci/mmol), poly(dA), and oligo(dT) were purchased from GE Healthcare Bio-Science Co. (Buckinghamshire, UK). All other reagents were of analytical grade and were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Preparation of dehydroaltenusin and its derivatives. Dehydroaltenusin and altenusin were synthesized chemically according to a previously reported method (7,8). Acetyl-dehydroaltenusin was prepared by acetylation of dehydroaltenusin-C3. Demethoxydehydroaltenusin and a 6H-benzo[c]-chromen-6-one derivative were newly synthesized starting from 2,2-dimethyl-4-oxo-4H-1,3-benzodioxin-5-yl trifluoromethanesulfonate and 4,5-bis(benzyloxy)-2-iodophenyl acetate, respectively. Altenuene and alternariol were isolated from the extract of Acremonium sp. (13). Demethyldehydroaltenusin, dehydroaltenusin-C3 derivative, dehydroaltenusin-C6 derivative and dehydroaltenusin-C12 derivative were prepared from a common intermediate, 7-hydroxy-2,2-dimethyl-5-(6-methyl-1,3-benzodioxol-5-yl)-4H-1,3-benzodioxin-4-one. All new synthetic products were fully characterized by 1H and 13C nuclear magnetic resonance (NMR), infrared spectroscopy (IR) and high resolution mass spectrometry (HRMS). The chemical structures of dehydroaltenusin derivatives are shown in Fig. 1. The synthetic details and characterization for all chemical derivatives will be reported elsewhere (Kuramochi et al, unpublished data).

DNA polymerases and other DNA metabolic enzymes. Pol α was purified from calf thymus by immuno-affinity column chromatography as described previously (14). Pol ß was purified from a recombinant plasmid expressing rat pol ß (15). The gene encoding the human pol γ catalytic subunit was cloned into pFastBac. The histidine-tagged enzyme was expressed using the BAC-TO-BAC HT Baculovirus Expression System according to the manufacturer’s instructions (Life Technologies, MD, USA) and purified using ProBoundresin (Invitrogen Japan, Tokyo, Japan) (16). Human pol δ and ε were purified by the nuclear fractionation of human peripheral blood cancer cells (Molt-4) using the second subunit of pol δ- and ε-conjugated affinity column chromatography, respectively (17). Recombinant human pol η and ι tagged with His₆ at their C-terminal ends were expressed in SF9 insect cells using a baculovirus expression system, and were purified from the cells as described previously (18,19). A truncated form of pol κ (i.e. hDINB1DC) with a 6 x His-tag attached at the C-terminus was overproduced using the BAC-to-BAC Baculovirus Expression System Kit (Gibco BRL) and purified as described by Ohashi et al (20).

![Figure 1. Chemical structures of dehydroaltenusin and its derivatives. (A) Core-modified dehydroaltenusin analogues. (B) Demethoxydehydroaltenusin derivatives modified at the 5-position.](image-url)
various concentrations and sonicated for 30 sec. Four micro-
derivative was dissolved in dimethyl sulfoxide (DMSO) at
nucleotide substrate, respectively. The dehydroaltenusin
described by Umeda
activity in the absence of the compound was taken as 100%.
The substrates of HIV-1 reverse transcriptase used were
nucleotide 5'-triphosphate, dNTP) substrate, respectively.
DNA template-primer and nucleotide (i.e., 2'-deoxyribo-
previously (25,26). Those for pol
fish pols, plant pols and prokaryotic pols were described
for demethoxydehydroaltenusin was greater than
that by dehydroaltenusin (i.e., the IC_50 value ratio against
demethoxydehydroaltenusin on pol ß was ~1.4-fold weaker
inhibitor than dehydroaltenusin. The inhibitory effect of
acetyl-dehydroaltenusin was a >10-fold
weaker pol α inhibitor than dehydroaltenusin, and compounds
4-7 did not influence the activities of pol α and ß.

Results

Effects of dehydroaltenusin and its analogues on the activities of mammalian pols α and ß. First, the inhibitory
effect of chemically synthesized dehydroaltenusin (Fig. 1A) on mammalian pols α and ß was investigated. Dehydroaltenusin dose-dependently inhibited
the activity of calf pol α, with 50% inhibition observed at a
dose of 0.68 μM (Table I) and almost complete inhibition at
4 μM (7). Although we reported previously that the naturally
purified dehydroaltenusin from a fungus (Acremonium sp.)
was a specific inhibitor of mammalian pol α (6), this
compound was also effective at inhibiting rat pol ß with an
IC_50 value of 64 μM.

In the prepared dehydroaltenusin analogues 2-7, acetyl-
dehydroaltenusin 2 and demethoxydehydroaltenusin 3
inhibited pol α and ß activities (Table I), and especially
demethoxydehydroaltenusin was an ~3-fold stronger pol α
inhibitor than dehydroaltenusin. The inhibitory effect of
demethoxydehydroaltenusin on pol ß was ~1.4-fold weaker
than that of dehydroaltenusin; therefore, the specificity of pol α inhibition by demethoxydehydroaltenusin was greater than
that by dehydroaltenusin (i.e., the IC_50 value ratio against
pol ß/pol α for demethoxydehydroaltenusin was 371, and for
dehydroaltenusin, 94). Acetyl-dehydroaltenusin was a >10-fold
weaker pol α inhibitor than dehydroaltenusin, and compounds
4-7 did not influence the activities of pol α and ß.

Inhibitory effects of demethoxydehydroaltenusin derivatives on mammalian pols α and ß activities. Since the structural
difference between dehydroaltenusin 1 and demethoxy-
dehydroaltenusin 3 is the group at the 5-position of these
compounds, four modified groups were synthesized chemically at the 5-position, 8-11, as shown in Fig. 1B. Fig. 2A shows the inhibition dose-response curves of
demethoxydehydroaltenusin derivatives, and all compounds
(0-1 μM) dose-dependently inhibited the activity of calf pol α.

Recombinant His-pol λ was overexpressed and purified
according to the method described previously (21). Fish pols
α and δ were purified from the testes of cherry salmon
(Oncorhynchus masou) (22). Pol I (α-like) from a higher plant,
cauliflower in florescence, was purified according to
the methods outlined by Sakaguchi et al (23). Recombinant rice
(Oryza sativa L. cv. Nipponbare) pol λ tagged with His6 at
the C-terminal was expressed in E. coli and purified from
the cells as described by Uchiyama et al (24). The Klenow
fragment of pol I from E. coli and human immunodeficiency
virus type-1 (HIV-1) reverse transcriptase were purchased
from Worthington Biochemical Corp. (Freehold, NJ, USA).
Taq pol, T4 pol and T4 polynucleotide kinase were purchased
from Takara (Kyoto, Japan). Purified human placental DNA
topoisoerasers I and IIa were obtained from TopoGen, Inc.
(Columbus, OH, USA). T7 RNA polymerase and bovine
deoxyribonuclease I were measured using standard assays
from Tamiya-Koizumi et al (28), Nakayama and Saneyoshi
(29), Mizushima et al (30), Solits and Uhlenbeck (31) and Lu
and Sakaguchi (32), respectively.

DNA polymerase assay. The reaction mixtures for pol α, pol ß,
fish pols, plant pols and prokaryotic pols were described
previously (25,26). Those for pol λ and pols δ and ε were as
were described by Umeda et al (16) and Ogawa et al (27),
respectively. The reaction mixtures for pols η, ι and κ were
the same as that for pol α, and the reaction mixture for pol λ,
was the same as that for pol ß. For pols, poly(dA)/
oligo(dT)_{12-18} (A/T=2/1) and ['^3H]-dTTP were used as the
DNA template-primer and nucleotide (i.e., 2’-deoxyribo-
nucleotide 5’-triphosphate, dNTP) substrate, respectively.
The substrates of HIV-1 reverse transcriptase used were
poly(rA)/oligo(dT)_{12-18} and dTTP as the template-primer and
nucleotide substrate, respectively. The dehydroaltenusin
derivative was dissolved in dimethyl sulfoxide (DMSO) at
various concentrations and sonicated for 30 sec. Four micro-
liters of each sonicated sample was mixed with 16 μl of each
enzyme (final 0.05 units) in 50 mM Tris-HCl (pH 7.5)
containing 1 mM diithothreitol, 50% glycerol and 0.1 mM
ethylenediamine tetraacetic acid (EDTA) and kept at 0℃ for
10 min. These inhibitor-enzyme mixture (8 μl) was added
to 16 μl of each enzyme standard reaction mixture and
incubated at 37℃ for 60 min, except for Taq pol, which was
incubated at 74℃ for 60 min. Activity without the inhibitor
was considered 100%, and the remaining activity at each
concentration of the inhibitor was determined relative to this
value. One unit of pol activity was defined as the amount of
enzyme that catalyzed the incorporation of 1 nmol of dNTP
(i.e., dTTP) into synthetic DNA template-primer in 60 min at
37℃ under the normal reaction conditions for each enzyme
(25,26).

Table I. IC_50 values of core-modified dehydroaltenusin
analogues on the activities of mammalian DNA polymerase α
and ß.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_50 value (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pol α</td>
</tr>
<tr>
<td>1</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>7.40</td>
</tr>
<tr>
<td>3</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>&gt;100</td>
</tr>
<tr>
<td>5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>6</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

1. Dehydroaltenusin; 2. acetyl-dehydroaltenusin; 3. demethoxy-
dehydroaltenusin; 4. altenuene; 5. 6H-benzo[c]chromen-6-one
derivative; 6. altenuene; 7. alternariol. These compounds were
incubated with calf pol α or rat pol ß (0.05 units each). Enzyme
activity in the absence of the compound was taken as 100%.
Pol was known inhibitor of replicative pols such as pols with that of dehydroaltenusin. Inhibition of demethoxydehydroaltenusin various pols and other DNA metabolic enzymes. Effects of demethoxydehydroaltenusin on the activities of later part of this study. Against other pol species and DNA metabolic enzymes in the study. Dehydroaltenusin was investigated for the inhibitory effects stronger than that of dehydroaltenusin; therefore, demethoxydehydroaltenusin was ~4-fold more effective against the activity of calf pol and other DNA metabolic enzymes in the same as that for pol β. On the other hand, demethoxydehydroaltenusin did not suppress other mammalian pols including pol λ; therefore, this compound could be a more potent pol α inhibitor than dehydroaltenusin.

Since aphidicolin exhibited inhibition of calf pol α with an IC_{50} value of 20 μM, the effect of demethoxydehydroaltenusin on this enzyme was ~83-fold stronger than that of aphidicolin (Table II). Aphidicolin also inhibited eukaryotic pol activities such as human pols δ and ε, fish (cherry salmon) pol α and δ, and plant (cauliflower) pol α, although dehydroaltenusin and demethoxydehydroaltenusin did not show any influence on these pols.

These three compounds had no inhibitory effect on human pols γ, η, ι and κ, prokaryotic pols such as the Klenow fragment of E. coli pol I (Klenow fragment), Taq pol and T4 pol, or other DNA metabolic enzymes such as calf primase of pol α, HIV-1 reverse transcriptase, T7 RNA polymerase, human DNA topoisomerases I and II, T4 polynucleotide kinase and bovine deoxyribonuclease I (Table II). The IC_{50} values for pols in Tables I and II did not change when the DNA template-primer was activated DNA (i.e., double-stranded DNA) digested by deoxyribonuclease I instead of poly(rA)oligo(dT)_{12-18}.

Demethoxydehydroaltenusin did not effect the thermal transition of melting temperature (data not shown); thus, none of the compound bound to double-stranded DNA, suggesting that this must inhibit enzyme activities by interacting with pol α directly. It was investigated whether an excessive amount of nucleic acid [i.e., poly(rC)] or protein [i.e., bovine serum albumin (BSA)] could prevent the inhibitory effect of demethoxydehydroaltenusin to determine whether the effect resulted from their non-specific adhesion to pol α or selective binding to specific sites. Poly(rC) and BSA had little or no influence on the effect of demethoxydehydroaltenusin, suggesting that the binding to pol α occurs selectively.

Dehydroaltenusin-C12 11 had the strongest inhibitory effect among the compounds tested, and in order of their effect, the derivatives were ranked as follows: dehydroaltenusin-C12 11 > dehydroaltenusin-C6 10 > dehydroaltenusin-C3 9 > demethoxydehydroaltenusin 3 > dehydroaltenusin 1 > demethyldehydroaltenusin 8.

On the other hand, the inhibitory effects on rat pol β activity by all the compounds were weaker than those on calf pol α, and the inhibitory effect of each derivative varied markedly in the following order: dehydroaltenusin-C12 11 > dehydroaltenusin-C6 10 > dehydroaltenusin-C3 9 > dehydroaltenusin 1 > demethoxydehydroaltenusin 3 > demethyldehydroaltenusin 8 (Fig. 2B). The IC_{50} value ratio against pol β/po α ranked as follows: demethoxydehydroaltenusin 3 (371) > dehydroaltenusin-C6 10 (267) > dehydroaltenusin-C3 9 (195) > dehydroaltenusin-C12 11 (180) > demethyldehydroaltenusin 8 (142) > dehydroaltenusin 1 (94). These results suggested that demethoxydehydroaltenusin 3 was the most specific pol α inhibitor of the synthesized derivatives, and the specificity of demethoxydehydroaltenusin was ~4-fold stronger than that of dehydroaltenusin; therefore, demethoxydehydroaltenusin was investigated for the inhibitory effects against other pol species and DNA metabolic enzymes in the later part of this study.

Effects of demethoxydehydroaltenusin on the activities of various pols and other DNA metabolic enzymes. The selective inhibition of demethoxydehydroaltenusin 3 was compared with that of dehydroaltenusin 1 and aphidicolin, which is a known inhibitor of replicative pols such as pols α, δ and ε (33). As shown in Table II, dehydroaltenusin and demethoxydehydroaltenusin were 94-fold and 371-fold, respectively, more effective against the activity of calf pol α than that of rat pol β. Dehydroaltenusin also inhibited human pol λ activity, and the inhibitory concentration on pol λ was almost the same as that for pol β. On the other hand, demethoxydehydroaltenusin did not suppress other mammalian pols including pol λ; therefore, this compound could be a more potent pol α inhibitor than dehydroaltenusin.

Since aphidicolin exhibited inhibition of calf pol α with an IC_{50} value of 20 μM, the effect of demethoxydehydroaltenusin on this enzyme was ~83-fold stronger than that of aphidicolin (Table II). Aphidicolin also inhibited eukaryotic pol activities such as human pols δ and ε, fish (cherry salmon) pol α and δ, and plant (cauliflower) pol α, although dehydroaltenusin and demethoxydehydroaltenusin did not show any influence on these pols.

These three compounds had no inhibitory effect on human pols γ, η, ι and κ, prokaryotic pols such as the Klenow fragment of E. coli pol I (Klenow fragment), Taq pol and T4 pol, or other DNA metabolic enzymes such as calf primase of pol α, HIV-1 reverse transcriptase, T7 RNA polymerase, human DNA topoisomerases I and II, T4 polynucleotide kinase and bovine deoxyribonuclease I (Table II). The IC_{50} values for pols in Tables I and II did not change when the DNA template-primer was activated DNA (i.e., double-stranded DNA) digested by deoxyribonuclease I instead of poly(rA)oligo(dT)_{12-18}.

Demethoxydehydroaltenusin did not effect the thermal transition of melting temperature (data not shown); thus, none of the compound bound to double-stranded DNA, suggesting that this must inhibit enzyme activities by interacting with pol α directly. It was investigated whether an excessive amount of nucleic acid [i.e., poly(rC)] or protein [i.e., bovine serum albumin (BSA)] could prevent the inhibitory effect of demethoxydehydroaltenusin to determine whether the effect resulted from their non-specific adhesion to pol α or selective binding to specific sites. Poly(rC) and BSA had little or no influence on the effect of demethoxydehydroaltenusin, suggesting that the binding to pol α occurs selectively.
These results suggested that demethoxydehydroaltenusin, which is a derivative of dehydroaltenusin, could be a selective inhibitor of mammalian pol $\alpha$.

**Discussion**

We previously reported an inhibitor of mammalian pol $\alpha$ from a fungus, *Acremonium* sp., and this compound was dehydroaltenusin $\text{1}$ (6). The inhibitory effect of dehydroaltenusin on pol $\alpha$ was 94-fold stronger than that on pol $\beta$, and 10 derivatives $\text{2-11}$ were prepared to screen for more potent pol $\alpha$ inhibitors than dehydroaltenusin.

As shown in Table I, compounds $\text{1-3}$ inhibited pol $\alpha$ activity, but compounds $\text{4-7}$ did not; therefore, the ketone group at the 5'-position (1 of Fig. 3), as contained by

---

Table II. IC$_{50}$ values of demethoxydehydroaltenusin derivatives modified at the 5-position on the activities of various DNA polymerases and other DNA metabolic enzymes.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>IC$_{50}$ value (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Mammalian DNA polymerases</td>
<td></td>
</tr>
<tr>
<td>Calf DNA polymerase $\alpha$</td>
<td>0.68</td>
</tr>
<tr>
<td>Rat DNA polymerase $\beta$</td>
<td>64</td>
</tr>
<tr>
<td>Human DNA polymerase $\gamma$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Calf DNA polymerase $\delta$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Human DNA polymerase $\epsilon$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Human DNA polymerase $\eta$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Human DNA polymerase $\iota$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Human DNA polymerase $\kappa$</td>
<td>69</td>
</tr>
<tr>
<td>Fish DNA polymerases</td>
<td></td>
</tr>
<tr>
<td>Cherry salmon DNA polymerase $\alpha$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Cherry salmon DNA polymerase $\delta$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Plant DNA polymerases</td>
<td></td>
</tr>
<tr>
<td>Cauliflower DNA polymerase $\alpha$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Rice DNA polymerase $\lambda$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Prokaryotic DNA polymerases</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> DNA polymerase I (Klenow fragment)</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td><em>Taq</em> DNA polymerase</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td><em>T4</em> DNA polymerase</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Other DNA metabolic enzymes</td>
<td></td>
</tr>
<tr>
<td>Calf DNA primase of DNA polymerase $\alpha$</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>HIV-1 reverse transcriptase</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td><em>T7</em> RNA polymerase</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Human DNA topoisomerase I</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Human DNA topoisomerase II</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td><em>T4</em> polynucleotide kinase</td>
<td>$&gt;100$</td>
</tr>
<tr>
<td>Bovine deoxyribonuclease I</td>
<td>$&gt;100$</td>
</tr>
</tbody>
</table>

1, Dehydroaltenusin; 3, demethoxydehydroaltenusin. These compounds were incubated with each enzyme (0.05 units). The enzymatic activity was measured as described in Materials and methods. Enzyme activity in the absence of the compound was taken as 100%.

---

Figure 3. The chemical structure of a dehydroaltenusin derivative, demethoxydehydroaltenusin 3, an inhibitor of mammalian pol $\alpha$. Groups (1) and (2) were essential for mammalian pol $\alpha$ inhibitory activity of the derivatives.
compounds 1-3 but not by compounds 4-7 in these analogues of dehydroaltenusin, must be essential for the inhibition. Since the specificity of pol α inhibitory activity of demethoxydehydroaltenusin 3 was higher than that of the other dehydroaltenusin derivatives (i.e., compounds 1 and 8-11) (Fig. 2), the group at the 5-position (2 of Fig. 3) could be important for the selective inhibition of pol species. These results show that the dehydroaltenusin derivative, which has no group at the 5-position (i.e., demethoxydehydroaltenusin 3) is the best inhibitor of mammalian pol α.

Demethoxydehydroaltenusin-induced inhibition of calf pol α activity was competitive with the template-primer and non-competitive with the dNTP substrate (data not shown). BIACore analysis demonstrated that this compound bound to the core domain of the largest subunit, p180, of mouse pol α, which has catalytic activity (34), but did not bind to the smallest subunit or the DNA primase p46 of mouse pol α (35) (data not shown). These results suggested that the demethoxydehydroaltenusin molecule competes with the template-primer molecule at its binding site on the catalytic domain of mammalian pol α, binds to the site, and simultaneously disturbs dNTP substrate incorporation into the template-primer. It was considered that demethoxydehydroaltenusin binds three-dimensionally in a special pocket in mammalian pol α. The structural relationship between the pol α catalytic subunit and demethoxydehydroaltenusin should be investigated by NMR analysis and computer simulation similarly to our previous studies using fatty acids and pol β (36-38). Such studies are currently in process.

Demethoxydehydroaltenusin will be used to elucidate the biochemical and biomedical functions of pol α from two different focal points; to understand the precise role of each pol in vitro, and to develop drug design strategies for cancer chemotherapy agents, as pol α is an essential enzyme for DNA replication and subsequently for cell division (1,2). Inhibitors of mammalian pol α are not only molecular tools useful for analyzing pols as a 'chemical knockout agent' instead of 'gene knockout method', but should also be considered as a group of potentially useful cancer chemotherapy agents. Demethoxydehydroaltenusin did not influence the activities of mammalian pols δ and ε, which are other replicative pols, but also showed no effect on pol α of another vertebrate, cherry salmon. Demethoxydehydroaltenusin is a type of antibiotic produced by a fungus and is chemically stable under in vivo conditions, indicating that it may be useful for analyzing the replication system within cells and for clinical use. Aphidicolin, once believed to be a pol α-specific inhibitor, is now known to also inhibit the activities of pols δ and ε (5). No pol α inhibitors with such a limited spectrum of action have been reported to date, and demethoxydehydroaltenusin can be a key agent for analyzing both the in vitro and in vivo functions of pol α in more detail. Thus, this compound can be a useful tool with which to study the in vitro functions of pol α and as a molecular probe to distinguish the structure of pol α.

Acknowledgements

This investigation was supported by a Grant-in-Aid for Research Fellowships for Young Scientists (I.K.), a Grant-in-Aid for Kobe-Gakuen University Joint Research (A.H.Y. and Y.M.) and a ‘Life Science Center for Cooperative Research’ Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology), 2006-2010 (H.Y. and Y.M.). Y.M. acknowledges a Grant-in-Aid for Young Scientists (A) (no. 19680031) from MEXT, Grants-in-Aid from the Nakashima Foundation (Japan), Foundation of Oil and Fat Industry Kaikan (Japan), and The Salt Science Research Foundation, no. 0853 (Japan).

References


