Sulfoquinovosyldiacylglycerol, KM043, a New Potent Inhibitor of Eukaryotic DNA Polymerases and HIV-Reverse Transcriptase Type 1 from a Marine Red Alga, *Gigartina tenella*

Keisuke OHTA,[a,b] Yoshiyuki MIZUSHINA,[a] Noriko HIRATA,[b] Masaharu TAKEMURA,[d] Fumio SUGAWARA,[a] Akio MATSUKAGE,[c] Shonen YOSHIDA,[d] and Kengo SAKAGUCHI*,[a]

Department of Applied Biological Science, Faculty of Science and Technology, Science University of Tokyo,[a] Noda-shi, Chiba 278–8510, Japan, 3rd Section of Research and Development, Toyo Suisan Kaisha, Ltd.,[b] Minato-ku Tokyo 108–8501, Japan, Laboratory of Cell Biology, Aichi Cancer Center Research Institute,[c] Nagoya-shi, Aichi 464–8681, Japan, and Laboratory of Cancer Cell Biology, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine,[d] Nagoya-shi, Aichi 466–8560, Japan.
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A new sulfolipid, KM043, which belongs to the 6-sulfo-α-D-quinovopyranosyl-(1→3′)-1′,2′-diacylglycerol (SQDG) class of compounds, has been isolated from a marine red alga, *Gigartina tenella*, as a potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type 1. Its structure was determined on the basis of spectroscopic and gas chromatographic analyses. The inhibition was dose-dependent, and complete (more than 90%) inhibition of DNA polymerase α (pol. α), DNA polymerase β (pol. β) and HIV-reverse transcriptase type 1 (HIV-RT) was observed at concentrations of 5, 10, and 30 μM, respectively.

Key words DNA polymerase; *Gigartina tenella*; inhibitor; sulfoquinovosyldiacylglycerol (SQDG)

Eukaryotic DNA polymerases are designated as α, β, γ, δ and ε, and each of them has different functions.[1,2] The roles of the polymerases have not yet been fully established, and therefore specific inhibitors would be helpful in studies to understand their precise roles *in vivo* and the factors controlling their activity.

Marine algae contain natural products which often show pharmaceutical activities.[3–5] We tried to isolate DNA polymerase inhibitors from sea algae, and found a new glycolipid KM043 from a marine red alga, *Gigartina tenella*. Its structure is quite similar to those of sulfonate- or sialic acid-containing glycolipids from cyanobacteria reported by Gustafson *et al.*[6] as AIDS-antiviral agents, and the compounds that were obtained from fern as eukaryotic DNA polymerase inhibitors.[7] By means of spectroscopic and chromatographic analyses, we showed KM043 to be a member of the sulfoquinovosyldiacylglycerol (SQDG) family, with the structure shown in Fig. 1. KM043 strongly and selectively inhibited DNA polymerase α (pol. α) , β (pol. β), and HIV-reverse transcriptase type 1 (HIV-RT).

In this paper, we report the isolation, structural determination and inhibitory activity towards DNA polymerases of KM043.

A red alga, *Gigartina tenella*, was collected at Sagami Bay, Kanagawa, Japan. The crushed dry alga (300 g) was extracted with 10 volumes of acetone. The extract was concentrated by evaporation and partitioned between EtOAc/water (3 : 1). The organic layer was evaporated to dryness and the residue was dissolved in EtOAc/MeOH/water (100 : 20 : 5), then subjected to silica gel column chromatography with the same solvent. Appropriate fractions, identified by biological assay, were pooled and concentrated. A part of the inhibitor was applied to a Waters HPLC system with an Octadesyl (ODS) column and eluted with CH_3CN/water (7 : 3). The active fraction was concentrated and applied to a C8 column eluted with CH_3CN/water (65 : 35). The active fraction was concentrated to obtain the pure KM043 (0.001%) fraction. KM043 was a colorless amorphous solid. The molecular formula $C_{45}H_{76}O_{12}S$ of the active inhibitor was determined by negative-ion HR-FAB-MS. The ^{1}H-NMR spectrum in CD_3OD showed seventy-two deuterium-unexchanged protons including twenty-four methylene protons at δ 1.29, ten olefinic protons at δ 5.40—5.30, and six methyl protons at δ 0.97 and 0.89. In the ^{13}C-NMR spectrum, two ester carbonyl carbon signals, ten olefinic carbon signals, two methyl carbon signals, and eight hydroxyl carbon signals associated with overlapped methylene carbon signals were observed. The α-anomer

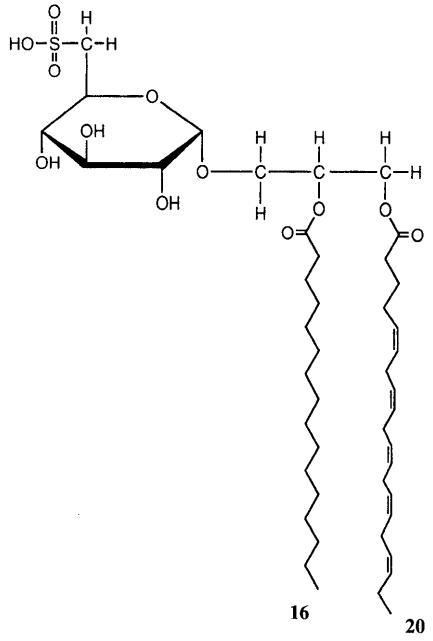

Fig. 1. Chemical Structure of the New 6-Sulfo-α-D-quinovopyranosyl-(1→3′)-1′,2′-diacylglycerol (SQDG), KM043

* To whom correspondence should be addressed.

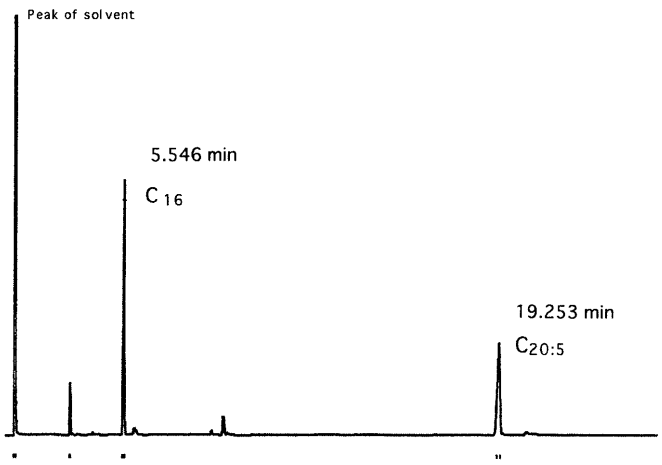

Fig. 2. Gas-Chromatographic Analysis of Fatty Acids in KM043

Operating conditions were as described in Experimental. Retention times of C_{16} and $C_{20:5}$ fatty acids are indicated. The ratio of C_{16} and $C_{20:5}$ fatty acids was 1 : 1.

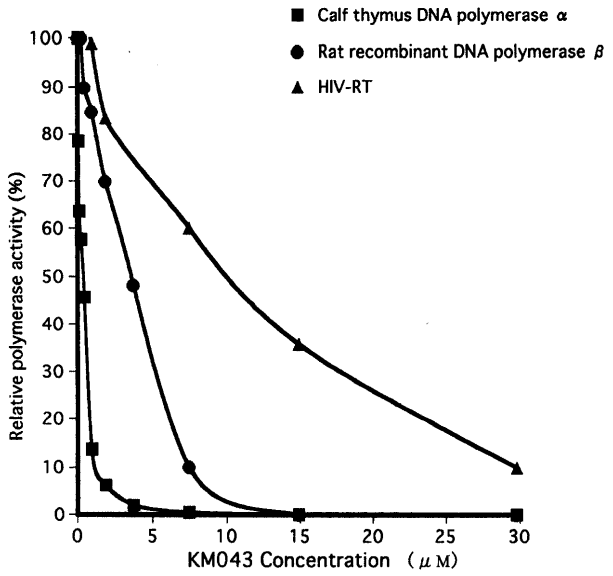

Fig. 3. Inhibition of DNA Polymerases α, β and HIV-RT by KM043

Each enzyme (0.05 U) was incubated with various concentrations of KM043 for 60 min at 37 °C.

was suggested by the carbon signal at δ 100.0 and the proton signal at δ 4.74 (d, J=3.6 Hz). The chemical shift of a signal at δ 54.3 was shifted upfield by sulfonation at C-6 of the carbohydrate moiety. By comparing the chemical shifts of this compound with those of sulfolipids, the compound was determined to belong to the family of sulfoquinovosyldiacylglycerides. After alkaline hydrolysis followed by methylation, methyl esters of palmitic acid ($C_{16:0}$) and 5(E), 8(E), 11(E), 14(E), 17(E)-eicosapentaenic acid ($C_{20:5}$) were identified by GLC analysis (Fig. 2). A predominant ion m/z 555 in the negative-ion FAB-MS was attributed to fragmentation at a primary ester, with loss of one of two fatty acids ($C_{20:5}$) in the glyceride moiety, and it indicated that palmitic acid is bound at the 2 position of glycerol backbone.[6] Furthermore the presence of a sulfo group was suggested by the loss of m/z 80 (SO_3^-) and 94 ($CH_2SO_3^-$) from the parental ion. Although the asymmetric centers of sulfoquinovose and glycerol were not determined, the optical rotation (+57.02°) of this compound was similar to those of other 1-(1′-*O*-α-D-sulfoquinovosyl)-diacyl-syn-glycerides. Thus, the structure of the inhibitor was determined as 1-(1′-*O*-α-D-sulfoquinovosyl)-2-palmitoyl-3-[5″(E), 8″(E), 11″(E), 14″(E), 17″(E)-eicosapentaenyl]-syn-glycerol.

KM043 exhibited inhibitory activity against pol. α, pol. β and HIV-RT, with IC_{50} values of 0.25, 3.6, and 11.2 μM, respectively (Fig. 3). The sulfo group in KM043 seems to be important for inhibitory activity.[8] The unsaturated long chain fatty acid (*i.e.*, eicosapentanoic acid) is also considered to be important for the inhibition.[7,9] KM043 showed cytotoxicity to Hela S3 at low concentrations (data not shown). Detailed analyses of the inhibition and bioactivity will be reported elsewhere.

Experimental

General Methods Optical rotation were determined on a JASCO DIP-SL. ^{13}C-, ^{1}H-NMR spectra was recorded on a GEMINI 300 spectrometer. The 49.8 and 3.30 resonances were those of CD_3OD. FAB-MS and HR-FAB-MS (glycerol matrix) were obtained on a JEOL HX-100 spectrometer. Gas chromatography for analysis of fatty acids containing KM043 was operated under following conditions: column, SPELCO WAX 0.25 mm × 30 m; Flow rate, 1 ml/min; FID detection; carrier gas, helium.

Extraction and Separation The crushed dry alga (300 g) was extracted with acetone (3000 ml) and the filtered solution was evaporated under reduced pressure. The extract was partitioned between EtOAc (800 ml × 3) and water (800 ml). The EtOAc-soluble portion was concentrated, applied to a silica gel column (Wako gel C-200; 4.0 × 40 cm) and eluted with EtOAc/MeOH/H_2O (100 : 20 : 5) to afford a fraction (300—450 ml), which was concentrated and applied to a C18 reversed-phase HPLC (YMC packed column D-ODS-5-ST; Yamamura Chemical Laboratories Co., Ltd.; 3.0 × 18 cm; flow rate, 4.0 ml/min; UV detection, 210 nm; eluent, CH_3CN/H_2O, 7 : 3). The active fraction was purified by C8 reversed-phase HPLC (Inertsil C8, GL Science Inc.; 0.5 × 25 cm; flow rate, 1.5 ml/min; UV detection, 210 nm; eluent, CH_3CN/H_2O, 65 : 35) to afford KM043 (3.1 mg, retention time 8.3 min).

KM043: A colorless amorphous solid; $[\alpha]_D$= +57.02° (c=0.11, MeOH) ^{13}C-NMR (CD_3OD, 75 MHz), δ 175.0, 174.9, 133.6, 132.8, 132.3, 130.0, 129.9, 129.3, 129.2, 129.1, 128.9, 128.2, 100.0, 75.0, 74.9, 73.5, 71.7, 69.8, 66.9, 64.3, 54.3, 35.2, 34.4, 33.1, 30.8—30.2, 29.6, 27.6, 26.9, 26.6, 26.0, 25.9, 23.7, 14.4. ^{1}H-NMR (CD_3OD, 300 MHz), δ 5.40—5.35 (10H, m), 5.32 (1H, m), 4.76 (1H, d, J=3.6 Hz), 4.50 (1H, dd, J=12.0, 2.9 Hz), 4.29 (1H, dd, J=6.6, 6.6 Hz), 4.18 (1H, dd, J=12.0, 6.8 Hz), 4.10 (1H, dd, J=10.9, 5.3 Hz), 4.05 (1H, dd, J=12.0, 2.0 Hz), 3.61 (1H, dd, J=10.1, 9.4 Hz), 3.56 (1H, m), 3.40 (1H, dd, J=5.2, 3.8 Hz), 3.36 (1H, m), 3.31 (1H, m), 3.08 (1H, dd, J=9.6, 9.1 Hz), 2.91 (1H, dd, J=14.4, 9.1 Hz), 2.80 (4H, m), 2.33 (4H, m), 2.12 (4H, m), 1.76 (1H, m), 1.66 (1H, m), 1.60 (4H, m), 1.48 (1H, m), 1.29 (24H, m), 0.97 (3H, t, J=7.5 Hz), 0.89 (3H, br t).

DNA Polymerase Assay Pol. α was purified from calf thymus by immuno-affinity column chromatography, as described previously.[10] Pol. β was purified from a recombinant plasmid expressing rat pol. β as described previously.[11] HIV-RT was purchased from Worthington Biochemical Corp.

For routine assay in the course of purification, recombinant rat pol. β were used. The standard reaction mixture for pol. β (final volume of 24 μl) contained 50 mM Tris–HCl (pH 8.8), 20 μM dTTP containing [^{32}P]dTTP (500 cpm/pmol), 215 mM $MgCl_2$, 15% glycerol, 150 mM KCl, 10 μg/ml of poly (dA), 5 μg/ml of oligo $(dT)_{12-18}$, 0.05 U of rat pol. β and various amounts of the fraction to determine the inhibitory activity, and reactions were performed at 37 °C for 60 min. One unit of pol. β catalyzes the incorporation of 1 nmol of dTTP into synthetic template-primer (poly(dA)/oligo$(dT)_{12-18}$, A/T=2/1) at 37 °C in 60 min.

The reaction mixture for HIV-RT-associated DNA polymerase activity was contained 50 mM Tris–HCl (pH 8.0), 5 mM $MgCl_2$, 15% glycerol, 130 mM KCl, 10 μg/ml poly (rA), 5 μg/ml oligo $(dT)_{12-18}$ used as template, and 10 μM dTTP containing [^{32}P]dTTP.

The conditions for pol. α assay were the same as for pol. β, except that KCl was omitted and the pH was 7.5. After incubation, the products

incorporating radioactive dTMPs were collected on DE81 filter paper as described,[12] and radioactivity was measured with a scintillation counter.

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