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Antimutagenic Activity of a Novel Ascorbic Derivative, Disodium Isostearyl 2-*O*-L-Ascorbyl Phosphate

Masayoshi HISAMA,*,[a] Sanae MATSUDA,[a] Hiroharu SHIBAYAMA,[a] and Masahiro IWAKI[b]

[a]*Central Research Center, Toyo Beauty Co., Ltd, 3–13–8, Higashinakamoto, Higashinari-ku, Osaka 537–0021, Japan, and* [b]*Department of Pharmacy, School of Pharmacy, Kinki University, 3–4–1 Kowakae, Higashiosaka City 577–8502, Japan*

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A novel amphiphilic vitamin C derivative, disodium isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS-2Na) possessing an alkyl chain of C_{18} to a stable ascorbate derivative sodium L-ascorbic acid 2-phosphate (VCP-Na), was synthesized and evaluated as an anti-mutagen with suppressive effect on SOS-inducing activity on mutagen in the *Salmonella typhimurium* TA1535/pSK1002 *umu* test. VCP-IS-2Na was assayed with chemical mutagens, *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (furylfuramide) and 4-nitroquinolin 1-oxide (4NQO), which do not require liver metabolizing enzymes. VCP-IS-2Na at a concentration of 0.40 μmol/ml suppressed 66.2%, 54.7% and 60.2% of the SOS-inducing activity on MNNG, furylfuramide, and 4NQO, and the 50% inhibitory dose value (ID_{50}) was 0.12 μmol/ml, 0.26 μmol/ml, and 0.17 μmol/ml, respectively. In addition, VCP-IS-2Na was assayed with 2-aminoanthracene (2AA) and 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), which require liver metabolizing enzymes. To study the structure-activity relationship, L-ascorbic acid (VC) and VCP-Na were also assayed with all mutagens. VCP-IS-2Na, suppressed the chemical and physical mutagens-induced SOS response greater than VC and VCP-Na in the *umu* test. Also, the antimutagenic activities of VCP-IS-2Na, VC, and VCP-Na against MNNG and Trp-P-1 were assayed by the Ames test using the *S. typhimurium* TA100 strain. In summary, this research suggests that VCP-IS-2Na showed potent antimutagenic effects against chemical mutagens and UV irradiation.

Key words——amphiphilic vitamin C derivative; disodium isostearyl 2-*O*-L-ascorbyl phosphate; antimutagenic activity; SOS response; *umu* test

INTRODUCTION

Cancer remains one of the most dreaded diseases even though methods for its detection at early stages and therapeutic remedies have greatly advanced. In Japan, cancer has been the leading cause of mortality since 1981.[1] Kee *et al.*[2] reported that the causes of cancer exist in the environment, especially in the diet and tabacco. Cancer is initiated by DNA damage, which is caused by natural and man-made chemical substances (mutagens) in the environment.[3]

Efforts in cancer chemotherapy have intensified over the past several decades, but many cancers still remain difficult to cure; therefore cancer prevention could become an increasingly useful strategy in our fight against this disease. Human epidemiology and animal studies have indicated that cancer risk may be modified by changes in dietary habits or dietary supplements. Humans ingest large numbers of naturally occurring antimutagens and anticarcinogens that may inhibit one or more stages of the carcinogenic process and prevent or delay the development of cancer. Recent studies indicate that compounds with antioxidant or antiinflammatory properties, as well as certain phytochemicals, can inhibit tumor initiation, promotion, and progression in experimental animal models. Epidemiological studies indicate that dietary factors play an important role in the development of human cancer. Attempts to identify naturally occurring dietary antimutagens and anticarcinogens may lead to new strategies for cancer prevention.

In the evaluation of the carcinogencity or mutagenicity of environmental chemicals, it is quite important to determine factors present in the environment that may affect these activities. With the development of laboratory techniques for the detection of possible environmental carcinogens and mutagens,[4] it has been shown that ordinary human diets contain several mutagens and antimutagens. In particular, the *umu* test system was developed to evaluate the genotoxic activities of a wide variety of environmental carcinogens and mutagens, using the expression of the SOS

*e-mail: hisama@toyobeauty.jp

genes to detect DNA-damaging agents in *Salmonella typhimurium*.[5,6] The system is based upon the abilities of carcinogens and mutagens to induce expression of an *umu* gene in *S. typhimurium* TA1535/pSK1002 in which a plasmid pSK1002 carrying a fused gene *umuC'-'lacZ* has been introduced; the *umu* gene seems to be involved in mutagenesis more directly than other known SOS genes.[7,8] The results of this test are also in agreement with the results of the Ames test and may be more useful with respect to simplicity, sensitivity, and rapidity.[9] Previously, antimutagenic effects of antimutagen have been reported by testing with the *umu* test system.[10,11]

L-Ascorbic acid, also known as vitamin C (VC) is known to be antioxidant, and has been widely used not only as a nutritional supplement but also pharmaceuticals and cosmetics in expectation of a certain pharmaceutical effects.[12] According to the peroxide oxidation theory, antioxidants act as antimutagens by chemically interacting with these products, as well as by decreasing the amount of peroxide compounds in the cell. The mechanism of this protection is believed to involve antioxidative ability to suppress free radical-generating processes. On the other hand, VC and ascorbate are reported to inhibit the formation of mutagenic *N*-nitroso compounds from nitrite and nitrate.[13,14] Thus, VC reduces the mutagenic activity of *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG) by chemically interacting with it,[15–17] as well as with 2-acetyl-aminofluorene,[18] ethylene methanesulfonate,[19] aflatoxin B_1,[20] dimethylnitrosamine, and other mutagens.[21]

The important physiological functions of VC are largely dependent on its unique 2,3-enediol moiety in the five-membered lactone ring, which exhibits strong electron-donating ability. However, its susceptibility to thermal and oxidative degradation together with its poor liposolubility make it difficult to maintain its physiological value over a long period of time and permeate through cell membrane. To solve these problems, L-ascorbic acid 2-phosphate (VCP-Na), a stable hydrophilic vitamin C derivative, has already been synthesized. However, this molecule still serves as a hydrophilic vitamin C derivative. Recently, we have synthesized a novel monoalkylated derivative of VCP-Na, sodium isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS-Na), with the aim of conferring efficient transdermal activity.[22] VCP-IS-Na has demonstrated satisfactory skin permeability in TESTSKIN™ LSE-high by using the vertical diffusion cells system.

In the present study, we assessed whether VCP-IS-2Na effectively suppresses the chemical and physical mutagens-induced SOS response in *S. typhimurium* TA1535/pSK1002. Additionally, the antimutagenic activity of VCP-IS-2Na against MNNG was investigated. The aim of this study was to clarify the potential of VCP-IS-2Na for prevention of the effects of mutagenicity, and to evaluate whether VCP-IS-2Na might be useful as a cancer chemopreventive agent.

MATERIALS AND METHOD

General Procedure ^{1}H- and ^{13}C-NMR spectra (δ, J in Hz) were recorded on a JEOL GSX 500 NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^{1}H-NMR spectra measured in chloroform-d_1. This solvent was also used for ^{13}C-NMR spectra. Infrared spectra (IR) were determined with FT/IR-470 Pulse Fourier Transform Infrared Spectrometer using a KBr disk. Fast atom bombardment high resolution mass spectra (FAB-HR-MS) were obtained on a JEOL JMS-HX 100 mass spectrometer. UV spectra were obtained on Shimadzu UV-2450 spectrophotometer. Optical rotations were measured by JASCO DIP-1000.

Materials L-Ascorbic acid was obtained from Sigma-Aldrich Co., Ltd. L-Ascorbic acid 2-phosphate sodium salt was obtained from Wako Pure Chemical Industries, Ltd. *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (furylfuramide), 4-nitroquinoline 1-oxide (4NQO), 2-aminoanthracene (2AA), and 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-b] indole (Trp-P-1) were purchased from Wako Pure Chemical Co. S9 (supernatant of 9000 g) and coenzyme, NADPH, NADH, and G-6-P were purchased from Oriental Yeast Co.

Synthesis of Disodium Isostearyl 2-*O*-L-Ascorbyl Phosphate (VCP-IS-2Na) Isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS) was synthesized from 5,6-*O*-isopropylidene-ascorbic acid and isostearyl dichlorophosphate by the methods from our previous report.[22] VCP-IS (1 mol) was reacted with sodium hydrate (2 mol) at 0°C for 3 h, ethanol was added, and evaporated in vacuo. The residue was subjected to recrystallization in acetonitrile and ethanol to give VCP-IS-2Na (65% yield) as a white powder. For VCP-IS-2Na; ^{1}H-NMR ($CDCl_3$): δ 0.88 (24H, t, J=

7.3, CH3–11, 13, 15, 17), 1.00–1.05 (2H, m, 12-H), 1.1–1.19 (2H, m, 16-H), 1.21–1.25 (4H, m, 9, 10-H), 1.26–1.33 (1H, m, 11-H), 1.47–1.53 (1H, m, 15-H), 1.76–1.82 (1H, m, 8-H), 3.81 (2H, m, 7-H), 3.95 (2H, d, J=7.0, 6-H), 4.12 (1H, dd, J=1.8, 7.0, CH-5), 4.81 (1H, d, J=1.8, 4-H), ^{13}C-NMR: 171.18 (C-1), 167.63 (C-3), 110.58 (C-2), 77.41 (C-4), 69.89 (C-7), 69.04 (C-5), 63.25 (C-6), 51.23 (C-12), 48.56 (C-16), 45.98 (C-8), 38.03 (C-10), 31.21 (C-13), 31.05 (C-17), 30.90 (C-14), 30.06 (CH_3-13), 29.99 (CH_3-13), 29.93 (C-18), 29.87 (CH_3-17), 29.75 (CH_3-17), 29.56 (C-11), 28.67 (C-9), 22.62 (C-15), 18.76 (C-11), 18.50 (C-19), IR (KBr, cm^{-1}): 3384, 2955, 1731, 1592, 1230, 1047, FAB-HR-MS m/z (rel. int.): 553.2540 ([M^+], $C_{24}H_{43}O_9PNa_2$), UV λmax (MeOH) nm (log ε): 241 (4.54), $[\alpha]^{D}_{24}$+12.4° (c=0.10, MeOH). VCP-IS-2Na was identified as disodium isostearyl 2-*O*-L-ascorbyl phosphate [disodium 2-(1,3,3-trimethyl-*n*-butyl)-5,7,7-trimethyl-*n*-octyl-L-ascorbyl phosphate] (Fig. 1).

***Umu* Test** The *umu* test for detecting the chemicals-induced SOS response was carried out according to the method of Oda *et al.*[5] using *S. typhimurium* TA1535/pSK1002, in which a plasmid (pSK1002) carrying a fused gene (*umuC*'-'lacZ) has been introduced. The overnight culture of bacterial strain was diluted 50-fold into TGA medium (1% Bactotryptone, 0.5% NaCl, and 0.2% glucose; supplemented with 20 mg/l ampicillin) and incubated at 37°C until the density reached 0.25–0.30 in OD_{600}. The bacterial culture was subdivided into 2.1 ml portions in test tubes, and the test compound (50 μl, diluted in 0.1M phosphate buffer), 0.1 M phosphate buffer (50 μl, pH 7.4), and mutagens MNNG (50 μl, 200 μg/ml in dimethyl sulfoxide (DMSO)), furylfuramide (50 μl, 2 μg/ml in DMSO), 4NQO (50 μl, 20 μg/ml in DMSO), activated Trp-P-1 (50 μl, 10 μg/ml in DMSO). In case of 2AA (50 μl, 20 μg/ml in DMSO) and Trp-P-1 (50 μl, 40 μg/ml in DMSO), 300 μl of S9-metablizing enzyme mixture including the cofactors was added instead of the phosphate buffer. As positive control, an equivalent volume of phosphate buffer was added instead of the test compound, whereas with negative control an equivalent volume of phosphate buffer and DMSO was added instead of both the test compound and the mutagen. After 2 h of incubation, at 37°C with shaking, the culture was centrifuged (3000 rpm) to collect cells, the SOS-inducing potency was estimated by measurement of *umu* operon expression in terms of cellular β-galactosidase activity. The unit of β-galactosidase activity was calculated according to the method of Miller.[23]

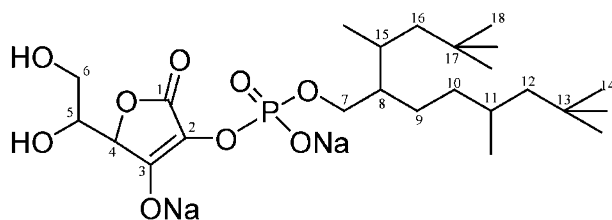

Fig. 1. Structure of Disodium Isostearyl 2-*O*-L-Ascorbyl Phosphate (VCP-IS-2Na)

Preparation of Activated Trp-P-1 Preparation of activated Trp-P-1 was carried out according to the method of Arimoto *et al.*[24]

UV Irradiation The cultures of the tester bacterial strain (*S. typhimurium* TA1535/pSK1002) were collected by centrifugation and suspended in 5 ml of 0.1 M phosphate buffer. The cell suspensions were then poured into Petri dishes and exposed to UV light (4.0 J/m^2) for 20 s using a germicidical lamp at room temperature.

Ames Test The mutation test was carried out according to the preincubation method,[25] which is a modification of the Ames method.[4]

RESULTS

Suppression of Chemical Mutagens-induced Responses by Disodium Isostearyl 2-*O*-L-Ascorbyl Phosphate (VCP-IS-2Na) Disodium isostearyl 2-*O*-L-ascorbyl phosphate (VCP-IS-2Na), L-ascorbic acid (VC), and sodium L-ascorbic acid 2-phosphate (VCP-Na) were evaluated in the *umu* test. VCP-IS-2Na, VC, and VCP-Na exhibited inhibitions on the MNNG-, furylfuramide-, and 4NQO-induced SOS response (Table 1). VCP-IS-2Na at a concentration of 0.40 μmol/ml suppressed 66.2%, 54.7% and 60.2 of the SOS-inducing activity on MNNG, furylfuramide and 4NQO, respectively, and the ID_{50} (50% inhibitory dose) values of VCP-IS-2Na were 0.12 μmol/ml, 0.26 μmol/ml, and 0.17 μmol/ml, respectively (Fig. 2). The suppressive effect of VCP-IS-2Na on MNNG is similar to that observed in the case of furylfuramide and 4NQO. These compounds were also assayed with 2AA and Trp-P-1, which require liver metabolic activation (Table 2). VCP-IS-2Na at a concentration of 0.40 μmol/ml suppressed 77.8% and 80.4% of the SOS-inducing activity on 2AA and

Table 1. Suppressive Effect of VCP-IS-2Na, VC, and VCP-Na on MNNG[a], Furylfuramide[b] and 4NQO[c] Using *S. typhimurium* TA1535/pSK1002

Mutagen	Compd	Control	Dose response[d] (μmol/ml)				
			0.4	0.2	0.1	0.05	0
MNNG	VCP-IS-2Na	104.8 (±2.2)	*286.0 (±5.9)	*319.6 (±3.6)	*386.2 (±4.1)	*451.8 (±3.4)	640.2 (±7.3)
	VC	104.8 (±2.2)	*465.3 (±3.5)	*482.9 (±10.7)	*518.1 (±4.4)	*566.5 (±8.8)	640.2 (±7.3)
	VCP-Na	104.8 (±2.2)	*493.0 (±7.9)	*512.1 (±3.6)	*546.5 (±2.9)	*588.7 (±7.7)	640.2 (±7.3)
Furyl-furamide	VCP-IS-2Na	84.9 (±8.5)	*373.0 (±3.6)	*418.5 (±6.5)	*487.7 (±3.9)	*581.9 (±6.8)	721.5 (±9.6)
	VC	84.9 (±8.5)	*590.7 (±8.5)	*636.9 (±10.3)	*661.0 (±4.9)	*686.6 (±11.3)	721.5 (±9.6)
	VCP-Na	84.9 (±8.5)	*621.0 (±5.9)	*652.9 (±4.3)	*665.4 (±2.2)	*688.5 (±8.0)	721.5 (±9.6)
4NQO	VCP-IS-2Na	94.3 (±6.6)	*359.9 (±3.6)	*402.5 (±5.8)	*484.6 (±4.7)	*587.7 (±7.1)	761.2 (±6.4)
	VC	94.3 (±6.6)	*593.2 (±6.1)	*643.9 (±5.2)	*675.5 (±6.6)	*702.8 (±9.0)	761.2 (±6.4)
	VCP-Na	94.3 (±6.6)	*635.7 (±3.2)	*663.6 (±3.8)	*679.5 (±5.3)	*714.7 (±10.9)	761.2 (±6.4)

[a] MNNG (200 μg/ml in DMSO) was added at 50 μl. [b] Furylfuramide (2 μg/ml in DMSO) was added at 50 μl. [c] 4NQO (40 μg/ml in DMSO) was added at 50 μl. [d] β-galactocidase activity (units). * $p<0.05$ when compared with controls.

Table 2. Suppressive Effect of VCP-IS-2Na, VC, and VCP-Na on 2AA[a], Trp-P-1[b] and Activated Trp-P-1[c] Using *S. typhimurium* TA1535/pSK1002

Mutagen	Compd	Control	Dose response[d] (μmol/ml)				
			0.4	0.2	0.1	0.05	0
2AA	VCP-IS-2Na	104.2 (±4.3)	*236.8 (±6.6)	*286.0 (±5.5)	*343.8 (±10.9)	*455.8 (±5.5)	701.8 (±5.6)
	VC	104.2 (±4.3)	*572.0 (±7.7)	*593.5 (±6.8)	*612.2 (±8.9)	*651.4 (±8.5)	701.8 (±5.6)
	VCP-Na	104.2 (±4.3)	*534.2 (±3.1)	*554.4 (±10.9)	*590.9 (±4.6)	*653.9 (±9.4)	701.8 (±5.6)
Trp-P-1	VCP-IS-2Na	99.3 (±3.4)	*204.1 (±4.9)	*229.7 (±7.1)	*285.9 (±5.3)	*374.3 (±6.4)	633.3 (±9.8)
	VC	99.3 (±3.4)	*496.4 (±5.9)	*514.7 (±7.2)	*535.1 (±6.8)	*572.1 (±10.9)	633.3 (±9.8)
	VCP-Na	99.3 (±3.4)	*468.8 (±4.6)	*484.9 (±7.2)	*512.9 (±4.5)	*552.6 (±7.9)	633.3 (±9.8)
Activated Trp-P-1	VCP-IS-2Na	91.7 (±5.2)	*412.2 (±3.3)	*434.3 (±5.6)	*460.4 (±2.3)	*504.1 (±5.1)	612.9 (±8.3)
	VC	91.7 (±5.2)	*557.9 (±6.2)	*567.1 (±8.8)	*579.0 (±12.6)	*590.1 (±8.2)	612.9 (±8.3)
	VCP-Na	91.7 (±5.2)	*535.2 (±5.3)	*554.4 (±9.8)	*568.2 (±4.5)	*586.0 (±6.7)	612.9 (±8.3)

[a] 2AA (20 μg/ml in DMSO) was added at 50 μl. [b] Trp-P-1 (40 μg/ml in DMSO) was added at 50 μl. [c] Activated Trp-P-1was added at 50 μl. [d] β-galactocidase activity (units). * Significant at $p<0.05$.

Trp-P-1, respectively, and the ID_{50} values of VCP-IS-2Na were 0.07 μmol/ml and 0.06 μmol/ml, respectively (Fig. 3). The suppressive effect of VCP-IS-2Na on 2AA is similar to that observed in the case of Trp-P-1. As these results of the umu test, the suppressive effects of VC on all chemical mutagens are similar to the suppressive effects observed in the case of VCP-Na, and VCP-IS-2Na had stronger suppressive effects on all chemical mutagens than VC and VCP-Na.

Suppressive Effect of VCP-IS-2Na on Metabolic Activation of Trp-P-1 The suppressive effects of VCP-IS-2Na, VC, and VCP-Na on metabolic activation of Trp-P-1 were determined by *umu* test. The value of β-galactosidase activity observed in the absence of VCP-IS-2Na, VC and VCP-Na was for activated Trp-P-1. As shown in Table 2 and Fig. 3, VCP-IS-2Na at a concentration of 0.40 μmol/ml suppressed 40.4% of the SOS-inducing activity on activated Trp-P-1. As these results of the *umu* test, the suppressive effect of VCP-IS-2Na, VC, and VCP-Na on activated Trp-P-1 was decreased compared with that of Trp-P-1.

Suppressive Effects of VCP-IS-2Na on UV Irradiation The suppressive effects of VCP-IS-2Na, VC, and VCP-Na on UV irradiation-induced SOS response were determined using the *umu* test (Table 3). VCP-IS-2Na suppressed 30.5% of the SOS-inducing activity due to UV irradiation at a concentration of 0.40 μmol/ml, and VC and VCP-Na hardly exhibited inhibition on the UV irradiation-induced SOS response (Fig. 4).

Antimutagenic Activity of VCP-IS-2Na in the

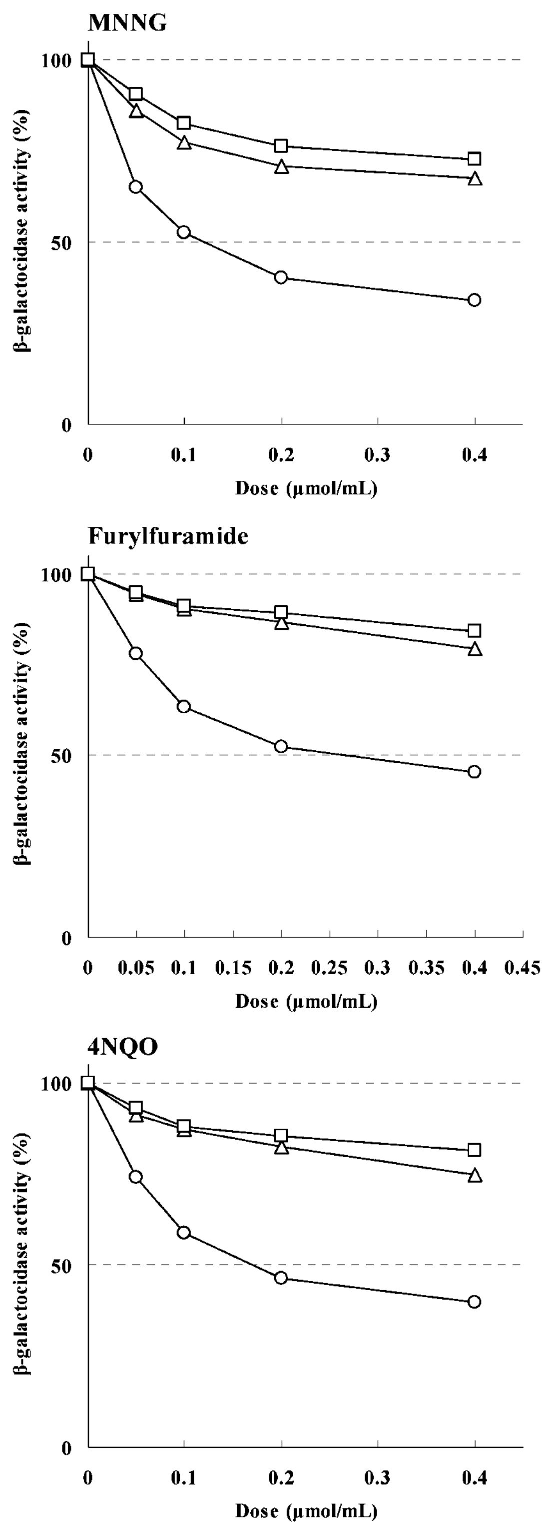

Fig. 2. Suppression of MNNG, Furylfuramide, and 4NQO-induced SOS Response by VCP-IS-2Na, VC, and VCP-Na in *S. typhimurium* TA1535/pSK1002

(○) Effect of VCP-IS-2Na; (△) effect of VC; (□) effect of VCP-Na. MNNG (200 μg/ml in DMSO) was added at 50 μl. Furylfuramide (2 μg/ml in DMSO) was added at 50 μl. 4NQO (40 μg/ml in DMSO) was added at 50 μl.

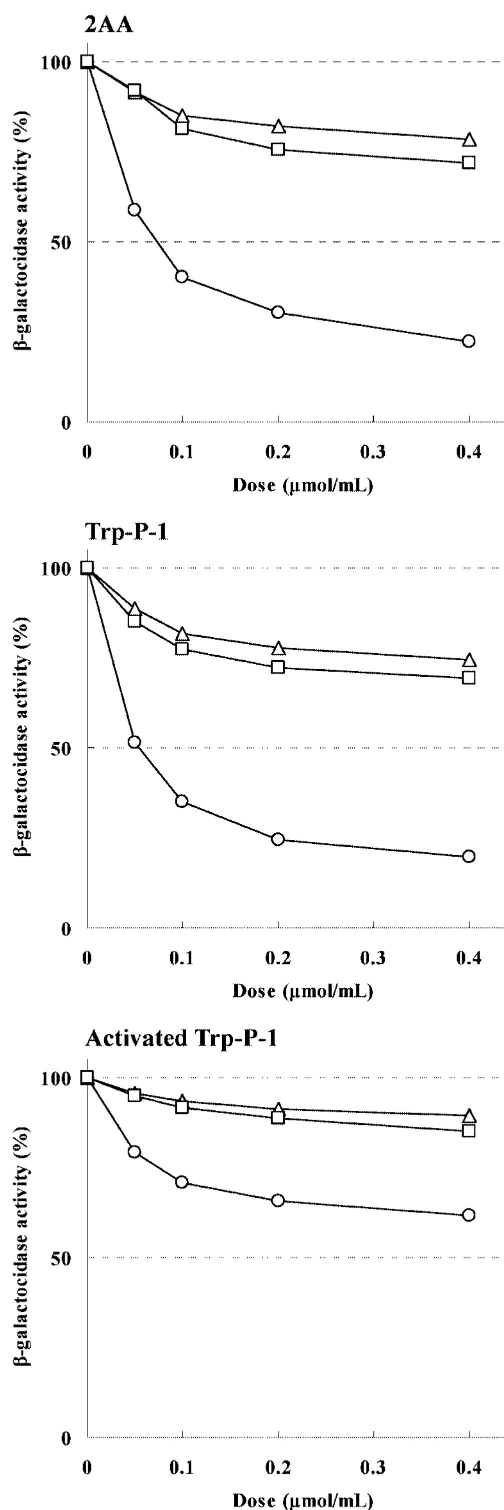

Fig. 3. Suppression of 2AA, Trp-P-1, and Activated Trp-P-1-induced SOS Response by VCP-IS-2Na, VC, and VCP-Na in *S. typhimurium* TA1535/pSK1002

(○) Effect of VCP-IS-2Na; (△) effect of VC; (□) effect of VCP-Na. 2AA (20 μg/ml in DMSO) was added at 50 μl. Trp-P-1 (40 μg/ml in DMSO) was added at 50 μl. Activated Trp-P-1was added at 50 μl.

Table 3. Suppressive Effect of VCP-IS-2Na, VC, and VCP-Na on UV Irradiation[a] Using *S. typhimurium* TA1535/pSK1002

Compd	Control	Dose response[b] (μmol/ml)				
		0.4	0.2	0.1	0.05	0
VCP-IS-2Na	98.9(±6.5)	*547.0(±7.8)	*567.8(±5.9)	*605.4(±5.3)	*668.9(±4.1)	743.8(±6.9)
VC	98.9(±6.5)	*680.8(±6.3)	*703.1(±4.6)	*721.5(±8.4)	733.6(±12.7)	743.8(±6.9)
VCP-Na	98.9(±6.5)	*669.6(±8.6)	*686.4(±11.6)	*714.3(±3.3)	*725.6(±6.5)	743.8(±6.9)

[a] The cells exposed to UV light (2.0 J/m^2) with a germicidal lamp at room temperature. [b] β-galactocidase activity (units). * Significant at $p<0.05$.

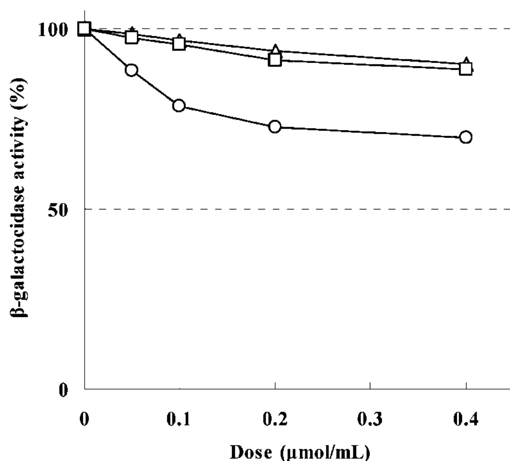

Fig. 4. Suppression of VCP-IS-2Na, VC, and VCP-Na on UV Irradiation in *S. typhimurium* TA1535/pSK1002

(○) Effect of VCP-IS-2Na; (△) effect of VC; (□) effect of VCP-Na. The cells were exposed to UV light (2.0 J/m^2) with a germicidal lamp at room temperature.

Ames Assay The antimutagenic activity of VCP-IS-2Na, VC, and VCP-Na against MNNG, Trp-P-1, and activated Trp-P-1 were also demonstrated by the Ames test using *S. typhimurium* TA100. VCP-IS-2Na at a concentration of 0.40 μmol/plate suppressed 82.2 % of the mutagenicity of MNNG, and the ID_{50} value of VCP-IS-2Na was 0.06 μmol/plate (Fig. 5). VCP-IS-2Na suppressed 77.4% of the mutagenicity of Trp-P-1 at concentration of 0.40 μmol/plate, and the ID_{50} value of VCP-IS-2Na was 0.08 μmol/plate (Fig. 5). On the other hand, these antimutagenic activities of VCP-IS-2Na against activated Trp-P-1 were remarkably decreased (Fig. 5).

DISCUSSION

VCP-IS-2Na showed suppressive effects on *umu* gene expression of the SOS response in *S. typhimurium* TA1535/pSK1002 against MNNG, furylfuramide, and 4NQO, which do not require liver metabolizing enzymes, and 2AA and Trp-P-1, which require liver metabolizing enzymes and UV irradiation. Moreover, according to our data VCP-IS-2Na has no toxic effects on the bacterial tester strain even at the concentration used in this test. Therefore the decrease of β-galactosidase activity cannot be due to the decrease of cell viability.

As for the structure-activity relationships, VCP-IS-2Na, VC, and VCP-Na had distinctly different suppressive potencies against the mutagens, according to their substitution patterns. As shown in Tables 1-3 and Figs. 2-4, VCP-IS-2Na had stronger suppressive potencies against all chemical and physical mutagens than VC and VCP-Na. As shown in Fig. 5, VCP-IS-2Na had stronger suppressive effects of the mutagenicity on all chemical mutagens than VC and VCP-Na. In contrast to VC and VCP-Na, VCP-IS-2Na is an amphiphilic compound with pronounced surface activity. It is readily soluble in lipids and consequently can be easily incorporated into the cell membrane phospholipid layer. This ability is likely to play an essential role in antimutagenesis of VCP-IS-2Na. VCP-IS-2Na is possibly incorporated into this layer of the membrane by its fatty acid chain, while its polar part, the VC residue, remains in the periplasm. Thus a coating consisting of VC residues is formed on the bacterial cytoplasmic membrane. It is well known that the greater part of detergents is incorporated into biomembranes and increases their permeability,[26] so the inactivation of mutagen is likely to proceed through its interaction with these residues in the periplasm and not through a decrease in its penetration. These results indicate that the amphipathicity of VCP-IS-2Na is an important factor for suppressing the SOS-inducing activity on all chemical and physical mutagens.

VCP-IS-2Na, VC, and VCP-Na were examined for their ability to suppress the metabolic activation of Trp-P-1 by the cytochrome P450 contained in S9. As shown in Table 2 and Figs. 3 and 5, VCP-IS-2Na,

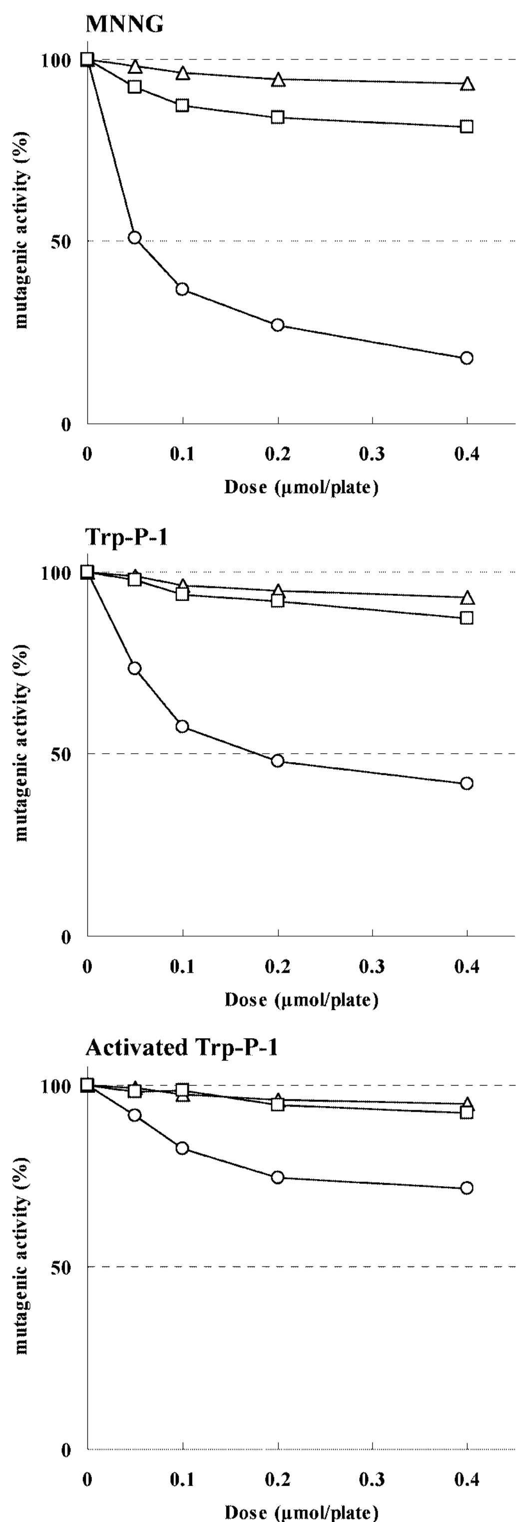

Fig. 5. Effect of VCP-IS-2Na, VC, and VCP-Na on the Mutagenicity of MNNG, Trp-P-1 and Activated Trp-P-1 in S. *typhimurium* TA100

(○) Effect of VCP-IS-2Na; (△) effect of VC; (□) effect of VCP-Na. MNNG (20 µg/ml in DMSO) was added at 50 µl/plate. Trp-p-1 (20 µg/ml in DMSO) was added at 50 µl/plate. Activated Trp-P-1was added at 50 µl/plate.

VC, and VCP-Na suppressed the weaker SOS induction and mutagenic activity on activated Trp-P-1 than on Trp-P-1. This result suggests the possibility that inhibition of the SOS-inducing activity and mutagenic activity on Trp-P-1, which was caused by VCP-IS-2Na, VC, and VCP-Na, was due to the inhibition of metabolic activation by the cytochrome P450 contained in S9.

VCP-IS-2Na had a suppressive effect on *umu* gene expression of SOS response in *S. typhimurium* TA1535/pSK1002 against UV irradiation (Table 3 and Fig. 4). The antimutagenic factors are divided into two main classes: one type, desmutagen, inactivates or destroys mutagens directly or indirectly out of the cell, and the other type of factor is called bioantimutagen, which suppresses the process of mutagenesis itself in the cells. From this result, the mechanism for inhibition of the SOS-inducing activity by VCP-IS-2Na may involve not only acted action on the mutagens but also involvement with cellular repair systems, and therefore VCP-IS-2Na might be a potent of bioantimutagen.

In the Ames test using *S. typhymurium* TA100, VCP-IS-2Na similarly inhibited the mutagenicity of MNNG, while VCP-IS-2Na showed a weak suppressive effect of the mutagenicity of Trp-P-1 compared with the *umu* test (Fig. 5). The difference between the results of the Ames test and the *umu* test may be caused by the differences of these methods. The principle of the *umu* test is based on the ability of DNA-damaging agents, most of which are potential mutagens and carcinogens, to induce the *umu* operon. A plasmid (pSK1002) carrying a fused gene (*umuC'-'lacZ*) was introduced into *S. typhimurium* TA1535. The strain TA1535/pSK1002 enabled us to monitor the levels of *umu* operon expression by measuring the β-galactosidase activity in the cells produced by the fusion gene. The Ames test is based on His^+ colonies as spontaneous His^+ revertant colonies, and the Ames tester strain TA100 carries a *uvrB* mutation. From the results of both the *umu* test and the Ames test, it may be expected that a correlation between the mutagen and VCP-IS-2Na involves different functions in both tests.

In summary, this research suggests that VCP-IS-2Na showed potent suppressive effects of SOS-inducing activity by chemical mutagens and UV irradiation. We expect that VCP-IS-2Na will be a useful cancer chemo-preventive agent. However, VCP-IS-2Na

may not exhibit its expected effects *in vivo* if it is adversely affected by factors such as absorption, biodisposition, and metabolism after its incorporation into the human body. Further studies with mammalian cells *in vitro* or *in vivo* are needed to determine the efficacy of VCP-IS-2Na for the prevention of human cancer.

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