Protective Effect of Sulfoquinovosyldiglyceride (SQDG) and Taurine Against Lipid Peroxidation

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ABSTRACT

Euglena gracilis Z grown under light is rich in sulfur-containing lipid, sulfoquinovosyldiglyceride (SQDG). However, the cells grown in the dark and its bleached mutant strain, SMZ that lacks chloroplasts does not accumulate as high SQDG content as *E. gracilis* Z grown under light. *E. gracilis* Z as a model of plant cell and *E. gracilis* SMZ as a model of animal cell were used to examine how they respond to UV-B irradiation stress. Only light-adapted *E. gracilis* Z was found to keep intracellular hydroperoxides level low even under UV-B exposure. We compared the antioxidative effect of SQDG and taurine both of which have common chemical structure as sulfonate group. Model experiments were made with water soluble initiator, 2,2′-azo-bis-(2-amidinopropane) dihydrochloride [AAPH] or UV-B irradiation with a peak at 312 nm in the presence of 3-hydroxykynurenine by using SQDG-embedded multilamellar liposomes or taurine-added liposome. Results showed that both in liposomes containing SQDG or taurine, AAPH-induced lipid peroxidation was significantly inhibited, but UV-B-induced lipid peroxidation was promoted in the SQDG-containing liposomes. Any significant effect was not recognized in the taurine-containing liposomes. Methylated SQDG also showed the same results as native SQDG, which suggest sulfonate group seems to have something to do with radical scavenging. In chloroplast, SQDG may play as a synergistic role for other antioxidants to prevent peroxidative damage in the light-adapted *E. gracilis* Z.

INTRODUCTION

Recently, it is one of the most widely accepted theory that the free radical causes aging and a species of diseases[1,2]. Free radical and/or reactive oxygen are inevitably brought forth in aerobic organisms by the environmental factor such as ionizing radiation, ultraviolet, smog, cigarette smoke or by the internal

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metabolic process, and they react with cellular components to lead to cellular damage and dysfunction of organella. To protect the hazardous effects of free radical and/or reactive oxygen, aerobic organisms have enzymatic and non-enzymatic antioxidant defense mechanisms such as superoxide dismutase, catalase, α-tocopherol, ascorbic acid and so on[3]. The thylakoid membrane of the chloroplasts in the photosynthesis is one of the most reactive oxygen rich tissue. Among various structural lipids in the thylakoid membrane sulfoquinovosyldiglyceride (SQDG) is present[4], and it constitutes about 10% of the membrane polar lipids[5], yet the role of SQDG in thylakoid membrane is little known. In the present paper, we report a protective role of SQDG in view of antioxidant mechanism by means of the fluorescence and chemiluminescence technique.

MATERIALS AND METHODS

＜Organism＞

Euglena gracilis Z, a wild strain and its bleached mutant (SMZ) were maintained in the light (2,800 lux) /dark cycle of every 12 hr as the light-adapted cells (Z-LD, SMZ-LD), or grown in the dark as the dark adapted cells (Z-D, SMZ-D). Each strain was cultured in Koren-Hutner medium[6] at 28°C for a week.

＜Assay of intracellular hydroperoxides in *E. gracilis* cells＞

Intracellular hydroperoxides in *E. gracilis* were assayed fluorimetrically with 2′,7′-dichlorofluorescin diacetate[7,8] (DCFH-DA). It was added to *E. gracilis* as the final concentration of 5 μM after UV-B irradiation, then the cell suspension was incubated for 5 min at 25°C to generate the substantial fluorescent 2′,7′-dichlorofluorescein (DCF) during enzymatic and/or heme oxidation. The fluorescence was detected for 5 sec. with SIT camera equipped to ARGUS-100 Microscope Image Analyzer, the intensity was quantitated by ARGUS-100 system.

＜Lipid compositions of Euglena cells＞

Lipids in *Euglena* cells were extracted by the modified Bligh and Dyer's method[9]. After separated into neutral lipids, glycolipid, phospholipids by column chromatography (Supelclean TM LC-Si SPE Tubes; Supelco, inc.), the compositions of lipids were determined by TLC-densitometric analysis.

＜Methylation of sulfonate group in SQDG＞

The sulfonate group in SQDG was methylated by diazomethane, which was prepared in diethyl ether solution by the action of KOH on N-methyl-N-nitroso-*p*-toluene-sulfonamide in ethanol[10].

＜Preparation of liposomes＞

Multilamellar liposomes were prepared by vigorous shaking of egg yolk phosphatidylcholine (EYPC) and subsequent ultrasonic irradiation. Finally 5 mM, dried EYPC under a N_2 stream was dispersed in 100 mM borate buffer (pH 9.3).

SQDG was preliminarily mixed with EYPC ranged from 1 to 10 mol% before N_2-dried, while taurine was preliminarily mixed with aqueous buffer ranged from 1 to 10 mM. Similarly, α-tocopherol (1 mol%), ascorbic acid (1 mM), histidine (1 mM) were preliminarily mixed before prepared liposomes, respectively.

＜Lipid peroxidation＞

Lipid peroxidation of EYPC-liposomes was induced by 2,2′-azo-bis-(2-amidinopropane) dihydrochloride [AAPH] or UV-B/3-hydroxykynurenine irradiation. AAPH was dispersed in the prepared liposomal suspensions to make 20 mM as the final concentration. The mixture was incubated at 37°C to initiate the radical reaction[11]. 3-Hydroxykynurenine as photosensitizer was dispersed in the prepared liposomal suspensions to make 10 μM as the final concentration, and the mixture was exposed to UV-B irradiation. UV-B, which was centered around 312 nm, was generated by CHROMATO-VUE® (UVP; california, U.S.A.). We made UV-B intensity, 0.4 mW/cm^2, which was the biological level reaching to our laboratory in summer.

＜Assay of lipid peroxidation＞

We assayed lipid peroxidation by chemiluminescence technique using isoluminol[12] as peroxide detector.

At designated time of incubation with AAPH or UV-B irradiation, liposomal suspensions were removed. Catalase solution was added to the liposomes at the final concentration of 12.5 U/ml, to quantitate lipid hydroperoxide selectively. After the reaction for 30 sec., the mixture of isoluminol and microperoxidase was added to the liposomes at final concentration of 6 μM and 3 mg/l, respectively.

The chemiluminescence measurement was made for 3 min. under high sensitive photon-counting camera, VIM-3 camera (Type C-2400) (Hamamatsu Photonics KK., Hamamatsu, Japan), equipped to Microscope Image Analyzer, ARGUS-100 system (Hamamatsu Photonics KK., Hamamatsu, Japan).

RESULTS AND DISCUSSION

＜Effect of UV-B irradiation on intracellular hydroperoxides in *E. gracilis*＞

As shown in Fig. 1., the level of DCF-fluorescence intensity was kept low only in *E. gracilis* Z-LD cell under UV-B-irradiation for 30 min. This result suggests that Z-LD cell has higher antioxidative potentials than three other cell types, i.e., SMZ-LD, Z-D, and SMZ-D, which inhibit the production of hyd-

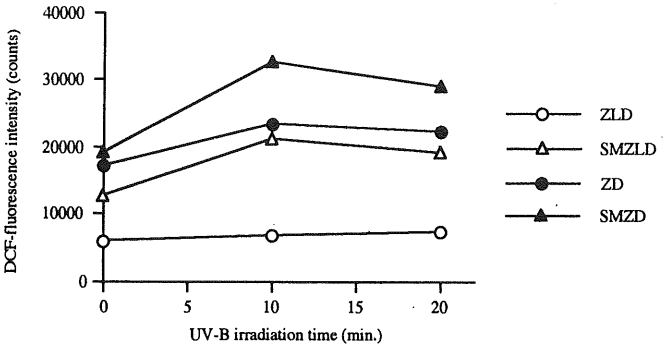

Fig. 1. Effect of UV-B irradiation on intracellular hydroperoxides in *Euglena gracilis*.
CDF: 2′,7′-dichlorofluorescein
Z-LD: the light-adapted cells of *Euglena gracilis* Z
SMZ-LD: the light-adapted cells of its bleached mutant
Z-D: the dark-adapted cells of *Euglena gracilis* Z
SMZ-D: the dark-adapted cells of its bleached mutant

roperoxides caused by UV-B irradiation.

＜Lipid compositions of Euglena cells＞

Table 1. shows that Z-LD cell is rich in glycolipids, however, the other cell types are rich in phospholipids. It is known that glycolipids are localized in the polar region in the chloroplast membranes. Judging from the fact that chloroplast is rich in reactive oxygen and present only in Z-LD cell, glycolipids are likely to participate to keep hydroperoxides caused by UV-B irradiation at low level in the Z-LD cell. So we focused on sulfur compound, sulfoquinovosyldiglyceride (SQDG) out of glycolipids, supposed that SQDG should have protective role against free radical and/or reactive oxygen-induced lipid peroxidation in the chloroplast. Therefore, we compared the antioxidant effect of SQDG and taurine, both of which have common chemical structure as sulfonate group and occur in oxidation-susceptible cells, on the lipid peroxidation by using multi lamellar liposomes.

＜Effect of SQDG and taurine on AAPH-induced lipid peroxidation＞

It is known that AAPH changes into a steady oxygen radicals due to its intramolecular thermal decomposition and it initiates a free radical chain reactions. It also oxidizes liposomal membranes from the outside.

To investigate the effect of SQDG and taurine on AAPH-induced lipid peroxidation, we estimated primary product, lipid hydroperoxide. As shown in Fig. 2, both SQDG and taurine demonstrated suppressive effect on the lipid peroxidation as the concentration increased. This result suggests that the sulfonate group seems to have something to do with the suppression of free radicals generation.

Table 1. Lipids classes of Euglena gracilis Z and SMZ under light and dark.

	*Euglena gracilis**)			
	Z-LD	SMZ-LD	Z-D	SMZ-D
Neutral lipid class				(%)
Hydrocarbon & Sterol ester	2.43	2.82	1.37	1.60
Wax ester	4.12	6.07	3.50	4.06
Fatty acid methyl ester	trace	0.67	0.36	0.42
Triglyceride	6.27	8.10	8.90	10.32
Free fatty acid	0.45	0.90	1.60	1.85
Sterol	0.88	1.23	3.13	3.63
Glycolipid class				
Monogalactosyldiglyceride (MGDG)	26.35	4.46	6.61	7.90
Digalactosyldiglyceride (DGDG)	22.19	1.17	1.62	1.43
Sulfoquinovosyldiglyceride (SQDG)	6.65	0.31	0.58	0.68
Phospholipid class				
Phosphatidylethanolamine	6.10	18.42	21.08	15.98
Phosphatidylglycerol	5.14	9.05	16.22	7.66
Phosphatidylcholine	16.98	43.18	31.88	39.24
Phosphatidylserine & Phosphatidylinositol	2.44	3.59	3.14	5.25

*) Abbreviation is the same as described in Fig. 1.

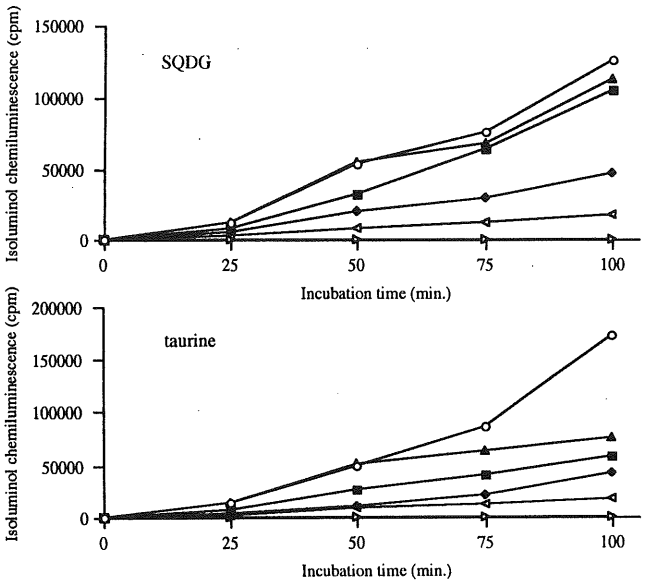

Fig. 2. Effect of SQDG and taurine on AAPH-induced lipid peroxidation

—○— control —▷— 1 mM ascorbic acid —◁— 1mol% α-tocopherol

—▲— 1mol% SQDG (1 mM taurine)

—■— 5mol% SQDG (5 mM taurine)

—◆— 10mol% SQDG (10 mM taurine)

<Effect of SQDG and taurine on UV-B-induced lipid peroxidation>

It is known that UV-B yields free radicals and active oxygen in the presence of photosensitizers in two main mechanisms; i.e., type I and type II photosensitization processes[13].

Similarlly as described for AAPH-induced lipid peroxidation, the effect of SQDG and taurine on UV-B-induced lipid peroxidation was investigated. As shown in Fig. 3, SQDG demonstrated promotive effect on the lipid peroxidation as the concentration increased, however, taurine didn't demonstrate any significant effect on the lipid peroxidation. Histidine played a role of negative control. This result suggests the reaction may be induced in a manner of singlet oxygen dependent oxidation and the unsaturated bonding of SQDG may be peroxidized predominantly.

<Effect of methylated SQDG on lipid peroxidation>

The sulfonate group in general has a low pK and, therefore, is ionized at physiological pH. To investigate the effect of the negative charge on AAPH or UV-B-induced lipid peroxidation, we methylated the sulfonyl group of SQDG, as shown in Fig. 4, and estimated its peroxidation in liposomal system as described above. As shown in Fig. 5, methylated SQDG demonstrated the suppressive effect on the AAPH-induced lipid peroxidation,however, it exhibited rather promotive effect on UV-B-induced lipid peroxidation, which were observed in the intact SQDG. This result suggests that the negative charge of

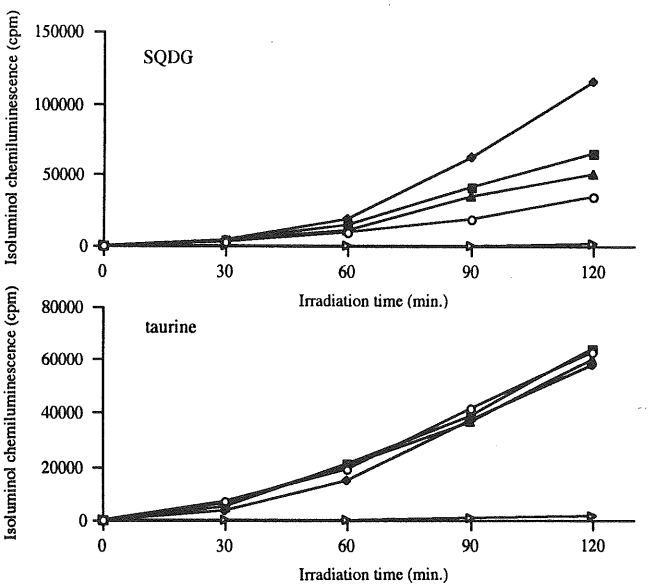

Fig. 3. Effect of SQDG and taurine on UV-B-induced lipid peroxidation
—○— control —▷— 1 mM histidine
—▲— 1mol% SQDG (1 mM taurine)
—■— 5mol% SQDG (5 mM taurine)
—◆— 10mol% SQDG (10 mM taurine)

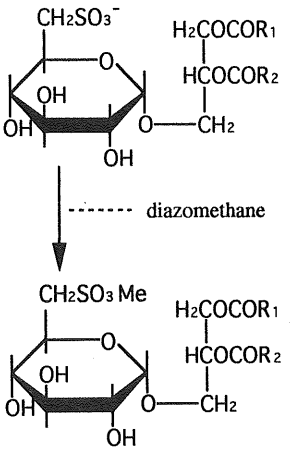

Fig. 4. Methylation of sulphonate group in SQDG.

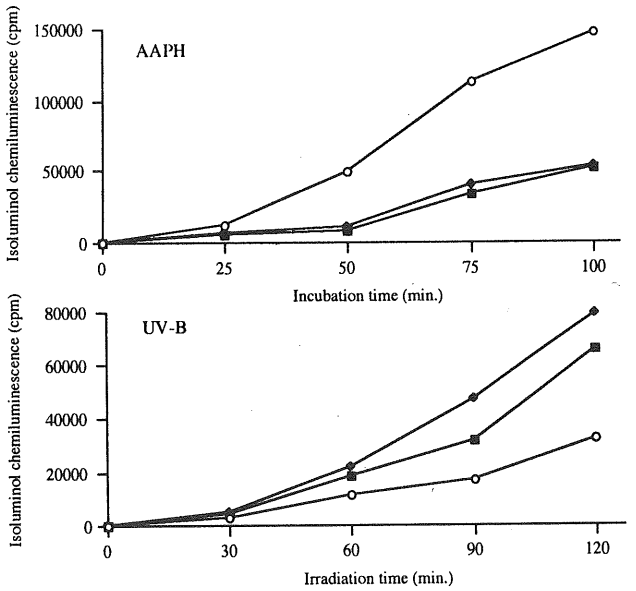

Fig. 5. Effect of methylated SQDG on AAPH and UV-B-induced lipid peroxidation
—○— control —◆— SQDG (10mol%)
—■— Methylated SQDG (10mol%)

sulfonate group has nothing to do with inhibition of lipid peroxidation.

Standing on the basis of results and discussion in the present study, we present a hypothesis that SQDG may participate in UV-B irradiated plant to make itself as a scape goat so as to protect other intracellular organs from reactive oxygen such as singlet oxygen. The singlet oxygen attacks the unsatu-

rated bonding of lipids, that results in lipid hydroperoxides. But in plant cell, SQDG may be peroxidized predominantly as compared with phospholipids which consitute cell membranes. the products, we call, SQDG hydoroperoxide may have high affinity to ascorbic acid peroxidase and keep intracellular hydroperoxides low level in plant cell.

It has been remained unknown why SQDG and taurine show suppressive effect on the radical chain lipid peroxidation. To reveal the inhibitory mechanism of those sulfur-containing compounds, further comparative experiments with non-sulfonate compounds such as monogalactosyldiglyceride (MGDG), digalactosyldiglyceride (DGDG) to SQDG, β-alanine, ciliatin to taurine should be necessary. On those experiments are we now under investigation.

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