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Formation of Artificial Lipid Membrane and their Photolysis in Mineral Water including Germanium

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We have attempted to determine the Germanium ion (Ge^{4+}) effect on the human body by observing the formation of artificial lipid membrane and photolysis in the mineral water containing Ge^{4+} ion. The artificial lipid membrane is prepared by using the phospholipid in the Germanium water and the formation efficiency of the liposomes is compared with those obtained in the plain mineral water without Ge^{4+} ion. This work shows that the liposomes are formed in the Germanium water better than in the non-Germanium water. The liposomes can be photolyzed by superoxide anion (O_2-˙) produced in the presence of some peptide such as NAT (N-acethyl-L-tryptophan). However, this is inhibited by superoxide dismutase (SOD), and it was found that the activity of SOD on the inhibition of the liposomes oxidative damage is higher in the Germanium water than in the non-Germanium water. It is concluded that the Ge^{4+} ion in mineral water helps the formation of new cell as well as elevation of SOD activity for the lipid oxidation.

key words: Germanium, superoxide (O_2-˙), superoxide dismutase (SOD), liposome, photolysis

INTRODUCTION

The effectuality and the results of clinical testing of Germanium, the one of the substances which is currently expected to make the dream of a world where no cancers or other geriatric diseases exist come true, have so far been disclosed a lot. And most of them have shown that Germanium can prevent of restrain cancers and other diseases of adult people. It is reported that when cells of human body are damaged, Germanium (socalled edible oxygen) penetrates and broadens Gap junction which blocked by carcinogens and finally normalize the function of Gap junction so that the cells are protected by being provided with sufficient amount of oxygen [1]. That is, Germanium is recognized as a new material which can keep superoxide (O_2–˙) from oxidizing cells and bringing about aging process and heal the incurable diseases or lessen the symptoms of those diseases.

Inorganic Germanium is unable to be taken by human, but organic one, which exists in plants such as ginseng and garlic or in the form of natural Germanium absorbed in water, can be taken by human [2,3]. So that a lot of research on physiological activity of Germanium are necessarily needed to carried out. However, there is not sufficient amount of research on the physiological activity now. Thus, in this study, the formation as well as the photodestructive rate of the biomimetic membrane, as artificial N-acethyl-L-tryptophan incorporated phospholipid membrane (liposomes) have been observed in the presence of different amount of Germanium in the aqueous solution. The activity of superoxide dismutase (SOD) that removes O_2–˙ has been observed by using different mineral waters containing different amount of Germanium.

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MATERIALS AND METHODS

In this experiment, the used Germanium water was generally offered by Taebaksansoo EumRyo, Inc. The natural Germanium examination has been reported in the 'RCH Research and Environmental Laboratories' report in the USA and 'Notice of the Ministry of Health and Welfare' in the Japan. As the result of analysis, they have shown that Germanium ion (Ge^{4+}) of 36.7 ~48.2 ppb exist in the form of adsorbed in water. For the comparison purpose, we selected the mineral water which does not contains Ge^{4+} ion that can be easily purchased in the market.

Materials. NaCl (99.5%) was purchased from Tedia Comapany, Inc. Tris(hydroxymethyl)aminomethane was obtained Wako Pure Chemical Industries, Ltd. Tris(hydroxymethyl)aminomethane hydrochloride (≥ 99%) was purchased from Fluka. N-acethyl-L-tryptophan (NAT), the photosensitizer was used as received from Research Plus Lab. Egg-lecithin (L-α-phosphatidyl-choline from egg yolk) was obtained from Sigma Chemcal Co.

Experimental. Liposomes were prepared by evaporating a chloroform solution of lipid to dryness in a round bottom flask under reduced pressure. NAT-incorporated liposomes were prepared by mixing a chloroform solution of egg-lecithin (0.4 mg/m*l*) with an ethanol solution of NAT (4 mg/m*l*) and evaporating to dryness with nitrogen gas followed by sonication in Tris-HCl buffer. Free NAT were eliminated by dialysis in pH 8 of buffer at 4°C for 24 hours [4].

The lipid was then dispersed in Tris-HCl buffer (0.1 M Tris-

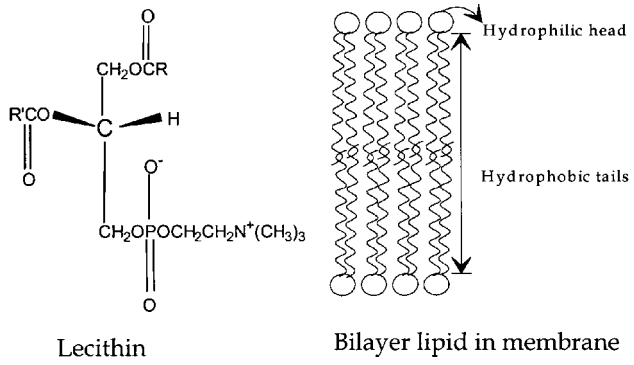
HCl+0.1 M NaCl) with NAT that Tris-HCl buffer made use of Germanium water and non-Germanium water by sonication at ambient temperature for 60 min with sonifier. The Germanium water of 1.8 L concentrated to 0.24 μg/m*l* Ge^{4+} ion and then it was diluted 0.12 μg/m*l* Ge^{4+} ion, 0.073 μg/m*l* Ge^{4+} ion using deionized water. Like this, the prepared containing Ge^{4+} ion displayed different pH in proportion to the increase of Ge^{4+} ion concentration. To adjust pH=8, all the various containing Ge^{4+} water sonicated in Tris-HCl buffer. Free NAT eliminated by dialysis in 8 of buffer at 4°C for 24 hours. Dialysis membrane was purchased from Membrane Filtration Products, Inc.

Samples of 2.5 m*l* were irradiated in UV cell located in front of water-cooled Pyrex vessel by using the light source, 450 W Xe lamp (KOKUSAN). The suspension of liposomes were continuously bubbled with oxygen gas via syringe needle. The relative liposome concentration was measured by turbidity at 750 nm on the UV absorption spectrum on a SCINCO UV S-2040 spectrophotometer. ORION 710A pH/ISE meter has been used to adjust pH of the solution.

RESULTS AND DISCUSSION

We have attempted to make artificial lipid membrane (liposomes) using α-phospholipid (so-called lecithin) in mineral water which contains Ge^{4+} ion or not. NAT was also incorporated into the liposomes. The lecithin is major constituent substance of brain and it has two long hydrophobic tails and hydrophilic head [5] (Scheme 1).

Initially, the suspension of liposomes, which contain artificial lipid membrane and NAT, show the turbidity at 750 nm, could be distinguished by naked eye. Before irradiation, each suspension of liposomes show quite a different scattering at 750 nm and it was summarized in Table 1. The turbidity was dramatically changed by the presences of Ge^{4+} ion or not, indicating that the formation of liposomes was significantly affected by Ge^{4+} ion. The turbidity increases with the relative amount of Ge^{4+} ion in the water (0.24 μg/m*l*>0.12 μg/m*l*>0.073 μg/m*l*). This is probably because Ge^{4+} ion existing near hydrophilic heads of lipid membrane increase the hydrophobic interaction as a result of increasing polar interaction between Ge^{4+} ion and water [6]. Thus, it should be suggested that the Ge^{4+} ion plays a role of the formation of artificial lipid membrane. Furthermore, in the high concentrated Ge^{4+} ion solution (≥ 0.12 μg/m*l*), the turbidity was much higher than in the low concentrated one (0.073 μg/m*l*), and other water solution, such as the plain mineral water solution and deionized water solution. This indicates that the concentration of Ge^{4+} ion also plays an important role of the formation of cells. This is consistent with the previous report that the morphology and structures in cells were affected by concentration of Ge^{4+} ion [7, 8].



Scheme 1.

Table 1. The initial turbidity of the prepared liposomes: in the presence of water different amount of Ge^{4+} ion under the anaerobic condition

	Turbidity at 750 nm
Ge water - 0.24 μg/m*l* Ge^{+4} ion	0.934 ± 0.018
Ge water - 0.12 μg/m*l* Ge^{+4} ion	0.617 ± 0.024
Ge water - 0.073 μg/m*l* Ge^{+4} ion	0.361 ± 0.007
Deionized water	0.493 ± 0.045
Plain mineral water	0.448 ± 0.018

To determine the effect of the concentration of Ge^{4+} ion on the destruction of cell, the photolysis of NAT-incorporated liposomes were also investigated by monitoring the decrease of the turbidity at 750 nm. Figure 1 shows the photolysis of liposomes as a function of irradiation time. The liposomes were most easily decomposed in the highly concentrated Ge^{4+} ion solution (0.24 μg/m*l*). As the concentration of Ge^{4+} ion decreased, the photodecomposition is slow down. In the lower concentrated solution, 0.073 μg/m*l*, the photodecompositive rate is about the same as in non-Ge^{4+} ion solution. Therefore, it is proposed that when the liposomes are exposed to the

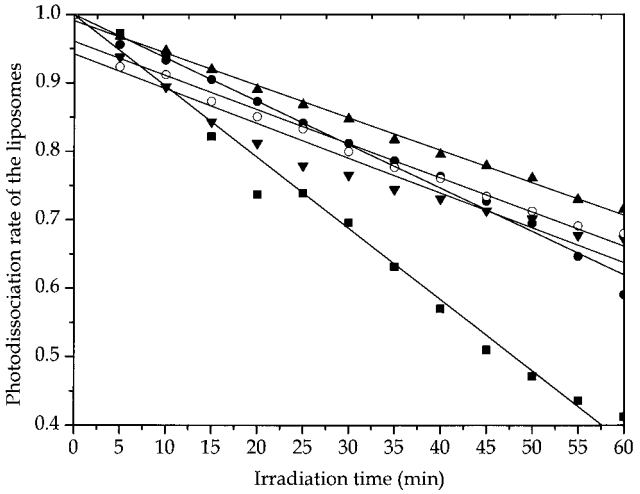


Figure 1. Photodissociation rate of the liposomes as a function of ≥ 320 nm irradiation in different water: (■) Ge water - 0.24 μg/m*l* Ge^{+4} ion, (●) Ge water - 0.12 μg/m*l* Ge^{+4} ion, (▼) plain mineral water, (○) deionized water, (▲) Ge water - 0.073 μg/m*l* Ge^{+4} ion.

Table 2. The turbidity of the prepared liposomes: in the presence of water different amount of Ge^{4+} ion under the aerobic condition

	Turbidity at 750 nm
Ge water - 0.24 μg/m*l* Ge^{+4} ion	0.678± 0.028
Ge water - 0.12 μg/m*l* Ge^{+4} ion	0.490± 0.018
Ge water - 0.073 μg/m*l* Ge^{+4} ion	0.291± 0.020
Deionized water	0.406± 0.052
Plain mineral water	0.312± 0.016

optimum concentrated Ge^{4+} ion (0.073 μg/m*l*), the formation of cells is relatively slow and the destruction of cells is also slow, indicating that the Ge^{4+} ion is a help to maintenance of cells. On the other hand, when the liposomes exposed to the highly concentrated Ge^{4+} ion (0.12–0.24 μg/m*l*), the formation of cells is fast, moreover, the destruction of cells is also fast by of Ge^{4+} ion [8].

As listed in Table 2, the turbidity was decreased even by the liposome solution with oxygen bubbling, indicating that the O_2 plays an important role in the destruction of cells as well as formation of cells. As reported by Yang *et al.*, Ge-132 (synthetic antioxidant germanium) enhances the activities of superoxide dismutase (SOD) in response to oxidative stress [9,10]. This suggested that Ge^{4+} ion might be proved beneficial in prevention of cell damages with a help of SOD. To understand the Ge^{4+} ion effects on the activity of O_2, SOD was added to artificial lipid membrane solution. Figure 2 shows the destructive rate of the liposomes by irradiation. The photodestructive rate was decreased further as compared to the dark situation under the aerobic condition by decreasing the concentration of Ge^{4+} ion from 0.24 μg/m*l* Ge^{4+} ion to 0.073 μg/m*l* Ge^{4+} ion. Furthermore, the activity of SOD was dramatically enhanced by Ge^{4+} ion, indicating that the Ge^{4+} ion plays a control factor of SOD activity. Thus, it should be suggested the formation and destruction of cells were strongly affected by Ge^{4+} ion and the activity of SOD was also controlled by Ge^{4+} ion.

CONCLUSION

It was investigated that the Ge^{4+} ion effect on the formation and the destruction of the cell lipid membrane. The formation and the destruction of cells were competitively occurred. The optimum concentration of Ge^{4+} ion was 0.073 μg/m*l*. It might be suggested that, if human drink the Germanium water, the

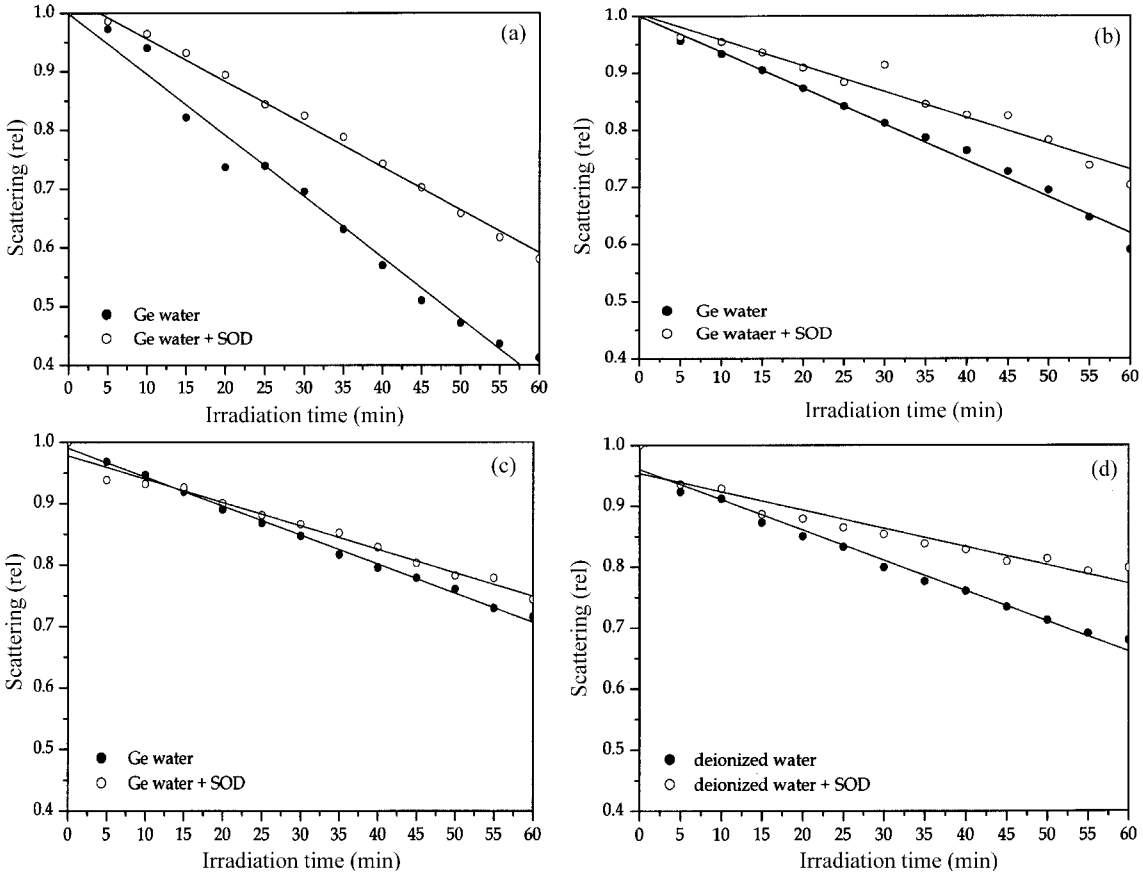


Figure 2. Photodissociation rate of the liposomes with and without superoxide dismutase in different water: (a) Ge water - 0.24 μg/m*l* Ge^{+4} ion, (b) Ge water - 0.12 μg/m*l* Ge^{+4} ion, (c) Ge water - 0.073 μg/m*l* Ge^{+4} ion, (d) deionized water.

oxidative damage (the aging process) of the cells by superoxide may be delayed.

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