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DESIGN AND DEVELOPMENT OF OXIDATIVE STRESS RESPONSIVE LIPOSOME MEMBRANE WITH ENZYMATIC ACTIVITY (LIPOZYME) AND ITS APPLICATION TO CHEMICAL/BIOSENSOR

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ABSTRACT

Superoxide dismutase (SOD) is inactivated by the H_2O_2 produced by its enzymatic reaction of superoxide anion. In the presence of neutral phospholipid liposomes, the inactivation of SOD was inhibited up to 5mM, suggesting that the liposomes might play role for the protection of SOD from H_2O_2 or the SOD-like function. In these phenomena, the secondary structure of SOD was lost in the interesting manner that liposome could maintain the SOD activity but not its structure. Based on these phenomena, we presented the concept "LIPOzyme" which is the novel terminology (liposome with the enzymatic activity).

KEYWORDS

Membrane Stress Biotechnology, Membrane Chip, Liposome, Amyloidogenic Protein

INTRODUCTION

Liposome is known to induce a variety of potential functions, such as chaperone-like function (Kuboi et al.1997, Yoshimoto and Kuboi, 1999), protein translocation (Umakoshi et al., 1998) and membrane fusion (Félix et al., 2002), under the stress condition (i.e. heating, acidification, and the reactive oxygen species (ROS)). LIPOzyme can herewith be defined as the liposome which can induce various kinds of enzyme-like functions under stress conditions, such as (a) the conformational change of polypeptides like as "molecular chaperone" and/or (b) the catalyst of the chemical reaction similar to "natural enzyme". Various kinds of LIPOzyme have previously been reported, such as (i) SOD-like functions using denatured SOD-liposome complex (Nagami et al., 2005) and Mn/Porphyrin-Liposome complex (Nagai et al., 2004) and (ii) metalloenzyme like function using $A\beta$ /Cu-liposome complex (Yoshimoto et al., 2005).

In this study, the interaction of liposome with SOD treated by ROS to form the LIPOzyme was investigated. Superoxide dismutase (SOD) has been known not only to degrade the oxygen radicals to hydroperoxide but also to be degraded by its enzymatic product based on the mechanism that the α -helix neighbouring to active site of SOD was broken. Liposome prohibited the decrease in enzymatic activity of SOD through the interaction between damaged SOD and liposome even under oxidative stress condition where α -helix was perfectly destroyed. Then, denatured SOD-liposome complex with SOD-like functions can be considered as a functional material to enzymatically degrade mild ROS. This LIPOzyme may permit us to bring about the interesting technology for a detection of ROS.

MATERIALS AND METHODS

Materials

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids (Albaster, AL, USA). Superoxide dismutase (bovine erythrocytes, 3700 U/mg; SOD), xanthine, xanthine oxidase and triphenyltetrazolium salt were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of analytical grade.

Liposome preparations

Large unilamellar vesicles (LUVs) were prepared by using the previous method (Kuboi et al., 1997). In brief, a desired quantity of POPC was dissolved into chloroform in a rounded bottom flask. The solvent was removed under vacuum using a rotary evaporator. This process was performed twice using chloroform and was pursued once more using diethyl ether in order to form a homogeneous thin layer of phospholipid on the wall of the flask. For the preparation of the liposome solution, an appropriate quantity of 100 mM Tris-HCl buffer was added into the round bottom flask containing the lipid film and, thereafter, five freezing-thawing cycles were applied before forcing the solution 15 times through a polycarbonate filter (100 nm in pore size) using an extruder device (Avestin, LiposoFast).

SOD activity

The xanthine/xanthine oxidase system was used as its indicator scavenger. The reduced triphenyltetrazolium salt was detected by measuring UV spectra at 450 nm. The relative SOD activity was defined as the ratio of the percentage inhibition of the triphenyltetrazolium salt reduction of SOD with hydrogen peroxide to that of intact SOD without hydrogen peroxide.

RESULTS AND DISCUSSION

Inactivation of SOD by hydrogen peroxide with and without liposomes

SOD inactivation under oxidative stress (with H_2O_2) was first investigated as shown in **Figure 1**. The incubation of 3 mM SOD with 5 mM H_2O_2 for 0-20 hrs at room temperature resulted in a time-dependent decrease in its enzymatic activity of decomposing superoxide anion to H_2O_2 and O_2 , measured by the modified nitro-blue tetrazolium method (**Figure 1a**). The activity of SOD was found to be lost at about 5 hrs. Figure 1b shows the SOD activities after 24 hrs of incubation with H_2O_2 at various concentrations (Fig.1b, open circles). At H_2O_2 concentrations higher than 5 mM, the SOD activities decreased to less than 20 % compared with the SOD activity in the absence of H_2O_2 . It has been reported that the sub-mM concentrations of H_2O_2 could be produced in a biological system under physiological conditions (Boveris et al., 1972) while SOD is normally produced in cells at the nM level (Aydin et al., 2004). The above results may suggest that the inactivation of SOD by H_2O_2 occurs at physiological concentrations.

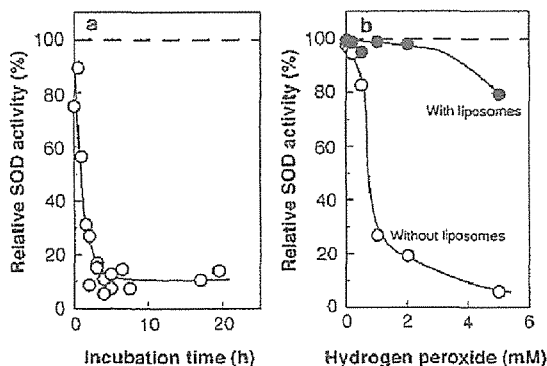


Figure 1 Oxidation of SOD by hydrogen peroxide with and without liposomes. (a) Time-course of inactivation of Cu,Zn-SOD during incubation with hydrogen peroxide in the absence of POPC liposome. (b) Effect of hydrogen peroxide concentration on SOD activity in the absence and presence of mM POPC liposome for 24 hrs.

To evaluate whether membranes have such a biological function in physiological systems, the inactivation of SOD by H_2O_2 was investigated in the presence of liposome composed POPC. **Figure 1b** shows the dependence of the SOD activity on the H_2O_2 concentration in the presence of the POPC liposomes (Fig.1b, closed circle). In the absence of liposomes, the SOD was completely inactivated at H_2O_2 concentration higher than 5 mM. Thus, a protective effect of liposomes against SOD inactivation was observed. The relationship between the liposome concentration and the SOD activity was also investigated at an H_2O_2 concentration of 2mM (**Figure 2**). The SOD activity increased with the POPC liposome concentration higher than 1.5 mM. It was thus found that the liposomes could protect SOD from oxidative inactivation in a concentration dependent manner and a concentration of 2 mM H_2O_2 was required for the complete protection against SOD inactivation under the conditions tested here. These results suggest the possibility that liposomes can remove H_2O_2 and protect SOD from H_2O_2 due to their antioxidant-like function and/or SOD-like function.

Relationship between the secondary structure of SOD and its activity

We address the α -helix content here because SOD has two helical domains in it. One helical domain is positioned close to active center (Ciriolo et al., 2001). The other helical domain hardly affects the SOD activity even though the quaternary structure was disrupted (Banci et al., 1998). In order to elucidate the principle of the above mechanism by liposomes, we checked the conformation of SOD in the absence and presence of liposomes as shown in **Figure 3**. In the absence of 2mM POPC liposome, the SOD activity drastically and step-wised decreased below 6 % of α -helix. On the other hand, almost SOD activity was kept in the presence of liposome nevertheless the content of α -helix. These results indicate that liposome can maintain the activity of SOD, but cannot protect its highly-ordered structure from H_2O_2 .

SOD-liposome complex as LIPOzyme

The damaged proteins can interact with lipid membrane through the hydrophobic interaction between them. Especially, the protein at molten-globule state easily and strongly interacts with lipid membrane to refold its own conformation (Kuboi et al., 1997, Yoshimoto and Kuboi 1999). The example of the refolding of carbonic anhydrase from bovine assisted by the liposome is the first attempt to our knowledge. In biological system, it is the protein that plays role for the refolding of the damaged and/or unfolded proteins. In such a sense, the above function can be considered as one of enzyme-like functions (*chaperone function*). Recently, the peptide/copper complex on liposome membrane has been reported to exhibit the metalloenzyme-like function (reductase) (Yoshimoto et al., 2005). In this study, the SOD-like activity could be shown under the oxidative stress condition.

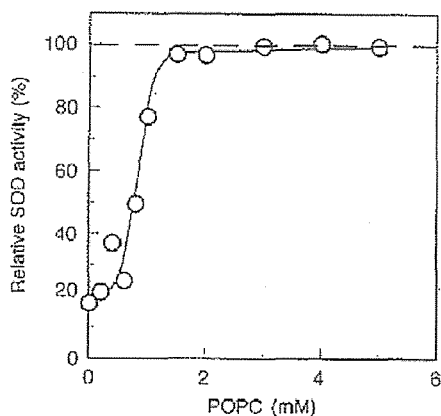


Figure 2 Effect of the addition of POPC liposome on inactivation of Cu,Zn-SOD. SOD activity was measured after 24 hr.

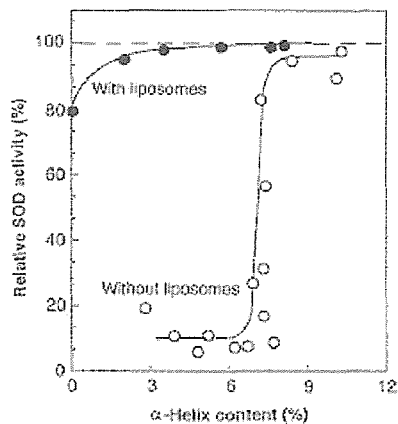


Figure 3 Relationship between relative SOD activity and α -helix content of Cu,Zn-SOD. In the absence (open key) or presence (closed key) of 2 mM POPC liposomes.

Although these approaches are just a part of Membrane Stress Biotechnology, *LIPOzyme* is the example for the development of “Membrane-Based Stress-Responsive Materials”.

CONCLUSIONS

In this study, systematic findings on the “membrane-based stress-responsive materials” namely *LIPOzyme* was successfully developed as exemplified degraded SOD-liposome complex. *LIPOzyme* is useful concept on the effective application of liposome to the practical field.

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