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Stabilization of quaternary structure and activity of bovine liver catalase through encapsulation in liposomes

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Abstract

Bovine liver catalase was encapsulated in an aqueous phase of the phospholipid vesicle (liposome) to improve the stability of its tetrameric structure and activity. The catalase-containing liposomes (CALs) prepared were 30, 50 and 100 nm in mean diameters (CAL_{30}, CAL_{50} and CAL_{100}, respectively). The CAL_{100} included the types I, II and III based on the amounts of catalase encapsulated. The CAL_{30}, CAL_{50} and CAL_{100}-I contained one catalase molecule per liposome, and the CAL_{100}-II and CAL_{100}-III on average 5.2 and 17 molecules, respectively. The storage stability of catalase in either CAL system was significantly increased compared to that of free catalase at 4 °C in a buffer of pH 7.4. At 55 °C, free catalase was much more deactivated especially with decreasing its concentration predominantly due to enhanced dissociation of catalase into subunits while it was so done at excessively high enzyme concentration mainly due to enhanced formation of catalase intermolecular aggregates. Among the three types of CAL_{100}, the CAL_{100}-II showed the highest thermal stability, indicating that an excess amount of catalase in the CAL_{100}-III was also disadvantageous to maintain an active form of the catalase even in liposome. In the CAL_{100}-III, however, the stability of catalase was significantly improved compared to that of free catalase at the same concentration. The CAL thermal stability was little affected by the liposome size as observed in the CAL_{30}, CAL_{50} and CAL_{100}-I. An intrinsic tryptophan fluorescence of the catalase recovered from the CAL_{100}-II thermally treated at 55 °C revealed that a partially denatured catalase molecule was stabilized through its hydrophobic interaction with liposome membrane. This interaction depressed not only dissociation of catalase into subunits but also formation of an inactive intermolecular aggregate between the catalase molecules in a liposome. Furthermore, either type of CAL_{100} showed a higher stability than free catalase in the successive decompositions of 10 mM H_2O_2 at 25 °C mainly because the H_2O_2 concentration was kept low inside liposomes due to the permeation barrier of the lipid membrane to H_2O_2.

Keywords: Bovine liver catalase; Liposomes; Thermal stability; Quaternary structure; Hydrogen peroxide decomposition

1. Introduction

Catalase (EC 1.11.1.6) is a redox enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and molecular oxygen [1]. Catalase is essential to protect aerobic organisms from toxic effects of H_2O_2. Bovine liver catalase consists of four identical subunits, and each of the four active sites contains a ferriporphyrin IX as a prosthetic group. The catalase is useful in various industrial fields such as food and textile processings which require control of H_2O_2 concentration under physiological conditions. The catalase is also utilized in an enzymatic oxidation reaction to depress the deactivation of the relevant enzyme and/or side reaction due to H_2O_2 produced [2,3]. Under practical reaction conditions, however, the catalase is readily deactivated, for example, by the shear force due to mechanical agitation in a solution and the gas–liquid interface in an aerated solution [3–5]. Such instability of the catalase as above is mainly attributed to a change in its quaternary structure which is known to be particularly unstable at its low concentrations [6,7]. In addition, an inactivating effect of H_2O_2 on catalase also limits practical application of the enzyme [1,8,9]. Therefore, much attention has been devoted to developing the catalase preparations which are applicable to a wide range of conditions for decomposing H_2O_2. The stability of catalase was improved using the immobilized whole cell catalyst [10] and

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additives [11], the cross-linked enzyme aggregates [12], and through immobilization of the enzyme in a rigid polymer matrix retaining sufficient active sites to H_2O_2 [13,14]. In particular, the immobilization of catalase followed by cross-linking of the subunits was reported to be effective to stabilize its quaternary structure [6,7]. The immobilized catalase showed a high stability because the diffusion resistance of H_2O_2 in solid support inhibits the catalase–H_2O_2 interaction. On the other hand, disadvantages of the immobilized catalase are considered to be enzyme deactivation during immobilization reaction as well as due to interaction with a hydrophobic interface [15], conformational change of the immobilized enzyme and physical stability of the support to the oxygen bubbles produced [14].

The enzyme reactions occurring in phospholipid vesicles (liposomes) have received considerable attention because both stability and reactivity of the liposomal enzyme are potentially regulated by keeping an intact molecular structure of the enzyme. The selective permeability of phospholipid bilayer membranes allows compartmentalization of biomacromolecules in liposomes as well as modulation of apparent selectivity of the liposomal enzyme to different substrates present in the liquid bulk [16]. In addition, some interactions of lipid membranes with enzyme molecules were reported to stabilize the enzymatic activity [17]. In immobilization of the liposomal enzyme through chemical bonding between liposome membranes and supports, no enzyme essentially underwent either deactivation or conformational change because the liposomal aqueous environment is rather independent of the environment outside the liposome [3]. We reported that the oxidation of glucose catalyzed by the liposomal glucose oxidase in the presence of liposomal catalase proceeded much more steadily than the reaction catalyzed by free enzyme systems [18]. As far as we know, however, there are a few studies [19] on the stability and activity of the liposomal catalase considering its tetrameric structure as well as the H_2O_2–catalase interaction.

In this work, the catalase-containing liposomes (CAL) prepared had a variety of the diameters and liposomal enzyme contents. The enzyme used was bovine liver catalase which was well-characterized in its molecular mass, amino acid sequence and conformational structure [20,21]. The liposomal membrane of the CAL was composed of POPC which has a well-defined chemical structure. In addition, the physicochemical aspects of the POPC liposomes have been well-characterized [22]. The liposomal catalase was characterized in terms of the stability and activity at two different temperatures of 4 and 55 °C and those in a prolonged decomposition of H_2O_2 at 25 °C with an emphasis on the tetrameric structure of catalase and its interaction with H_2O_2. The results obtained would ascertain that it might be a general strategy to stabilize multimeric enzymes through their liposomal encapsulation.

2. Materials and methods

2.1. Materials

Bovine liver catalase (hydrogen peroxide oxidoreductase, EC 1.11.1.6, 8000 U/mg, $M_r = 240{,}000$) and hydrogen peroxide (H_2O_2) solution were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was obtained from Avanti Polar Lipids Inc. (Alabaster, AL). 1-Anilinonaphthalene-8-sulfonic acid (ANS) was obtained from Molecular Probes (Eugene, OR). All reagents were used as received. Water used was purified and sterilized using a water purification system (Elix 3UV, Millipore Corp., Billerica, MA).

2.2. Preparation of catalase-containing liposomes (CAL)

POPC (40–60 mg) was dissolved in chloroform (4–6 mL) in a round bottom flask and the solvent was evaporated to form a lipid film. The POPC film was dissolved in diethylether and the solvent was removed. This procedure was performed twice. The residual solvent in the POPC film was removed under high vacuum (<8 Pa) using a freeze-dryer. Then the dry POPC film was hydrated at pH 7.4 with 50 mM Tris–HCl/100 mM NaCl buffer solution containing catalase at the concentrations of 1.3, 20 or 80 mg/mL. The multilamellar vesicles (MLVs) formed was frozen at −80 °C and thawed at 35 °C to enhance the transformation of small liposomes to larger ones. This freezing and thawing treatment was performed seven times. Negligible deactivation of catalase was observed by the successive treatment. The final MLVs were extruded through Nuclepore® polycarbonate membranes with nominal pore diameters of 100, 50 or 30 nm [22,23]. Extrusion through 100 nm membrane preceded extrusions with 50 and 30 nm membranes. The liposome suspension obtained was a mixture of the catalase-containing liposomes (CAL) and free catalase. The free catalase was separated from the CAL by passing the suspension through a sepharose 4B column. The CALs obtained as described above were denoted as CAL_{100}, CAL_{50} and CAL_{30} with the subscript standing for the mean diameter of liposomes approximately equal to the nominal pore size used in the final extrusion step [23]. There were three types of CAL_{100} (CAL_{100}-I, CAL_{100}-II and CAL_{100}-III) which were prepared by hydrating the dry lipid films with the Tris buffer containing catalase at the concentrations of 1.3, 20 and 80 mg/mL, respectively. The CAL_{30} and CAL_{50} were prepared with the buffer of 1.3 mg/mL catalase. Enzyme-free liposomes with nominal diameter of 100 nm were prepared in the same way as described above except that the buffer solution for the dry lipid hydration contained no enzyme. It should be noted that the POPC membranes (the main transition temperature T_m of -2.5 ± 2.4 °C [24]) are in the liquid-crystalline state or in the liquid-disordered phase under the present experimental conditions (4–55 °C).

2.3. Measurement of activities of liposomal and free catalase

The activity of catalase was measured with H_2O_2 as substrate as follows. For a CAL, aliquots of the CAL suspension were added to the Tris buffer solutions containing 10 mM H_2O_2 to give the overall catalase concentrations of 0.1–0.2 μg/mL. Then the time course of the decomposition of H_2O_2 was followed at 25 ± 0.3 °C based on the absorbance for H_2O_2 at 240 nm with the molar extinction coefficient of $\varepsilon_{240} = 39.4\ M^{-1}\ cm^{-1}$ [25] using a spectrophotometer (Ubest V-550DS, JASCO, Japan). The initial decomposition rate was taken as the apparent activity of the liposomal catalase. The intrinsic catalase activity of the CAL was measured in the same way as described above except that the reaction solution contained 10–40 mM cholate for complete solubilization of the CAL membranes. Cholate, POPC and their mixed micelles had negligible effect on the above activity measurements. The activity efficiency E for the CAL was defined as the apparent CAL activity measured with the prepared CAL relative to the intrinsic CAL activity determined as described above. The smaller value of E means that the CAL reaction rate is more strongly limited by the permeation of substrate H_2O_2 through liposome membranes. For free catalase, its activity was measured in the same way as above at the catalase concentrations of 0.1–0.2 μg/mL.

2.4. Measurement of ANS fluorescence

The ANS molecules are known to show intense fluorescence in liposome membranes [26,27]. The anionic ANS molecules are considered to be incorporated in the interfacial region of the membrane. In contrast, free ANS molecules in an aqueous solution show very low fluorescence. The ANS fluorescence in liposome suspensions was measured in the presence and absence