Electron Spin Resonance Study on Sugar Ester Nano Vesicles Entrapping Active catalase

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Abstract

Therapeutic application of enzymes demands the presence of a stable enzyme formulation. In this study, catalase enzyme (CAT) was entrapped in biocompatible biodegradable flexible sugar esters (SE) nano vesicles to improve the stability of its tetrameric structure and antioxidant activity. Thin film hydration method was used for enzyme entrapment in SE vesicles, where the formulation conditions used proved to preserve the fragile nature of the enzyme. In addition, electron spin resonance (ESR) was used to test the general hypothesis that vesicles composition, including lecithin and cholesterol influence the fluidity of vesicles bilayer and hence its flexibility. For this purpose the effect of phospholipids of different composition and the influence of different concentrations of cholesterol on vesicles bilayer fluidity was studied using ESR. Owing to the nondestructive nature of ESR measurements it is considered a well suited technique to monitor as well the encapsulated CAT inside vesicles without affecting its activity. Two different nitroxide spin probes were used, that is 16-doxyl stearic acid (16-DSA) for the study of the different dynamic properties of vesicles bilayer and 3-(2-iodoacetamido) proxyl (IAA) for ESR measurements of entrapped CAT in SE vesicles. The results obtained indicated the coexistence of two regions in the bilayer of the tested vesicles, one richer in cholesterol (and more rigid) and one with less cholesterol content (and more mobile). It was also concluded that CAT is not freely moving inside the vesicle rather its movement became restricted upon encapsulation inside the vesicle. This could account for the high stability observed for entrapped CAT compared to the free one. The flexible CAT nano SE vesicles developed in this study are considered to be a novel addition to Pharmaceutical industry arsenal for a safe active CAT topical application.

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