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A Liposome-Based Assay for Quantitation of Detergents

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Abstract

The amount of enzyme released from liposomes exposed to detergents varies with the amount of detergent present. This fact makes it possible to quantitate the detergent. Multilamellar liposomes containing entrapped peroxidase were adsorbed to paper discs and then exposed to solutions containing detergents. The assay procedure proved useful for assaying detergents down to their critical micelle concentration (CMC), and for an induced leakage of ionic detergents also below their CMCs.

Index Entries: Liposomes, in detergent assay; detergent, liposome assay of; assay, of detergents by liposomes; peroxidase, liposome entrapped; entrapped enzymes, in detergent assay.

Introduction

Biochemical systems can be used in the analysis of pollutants in the environment. These systems may either be used for identifying and quantifying a single substance (*1*), or the total biological effect of all the pollutants in water (*2*), for instance. In the measurement of the total biological effect, either living cells or organelles have been used. However, to stabilize the experimental conditions for quantitating detergents, liposomes were used to study their effect on biological membranes (Fig. 1). Liposomes were prepared with a slight modification of a previously published method (*3*). Phosphatidylcholine (7 μmol), dicetylphosphate (2 μmol) and cholesterol (1 μmol) were dissolved in chloroform in a glass Wifug test tube. The solvent was evaporated and a dry lipid mixture formed on the wall of the

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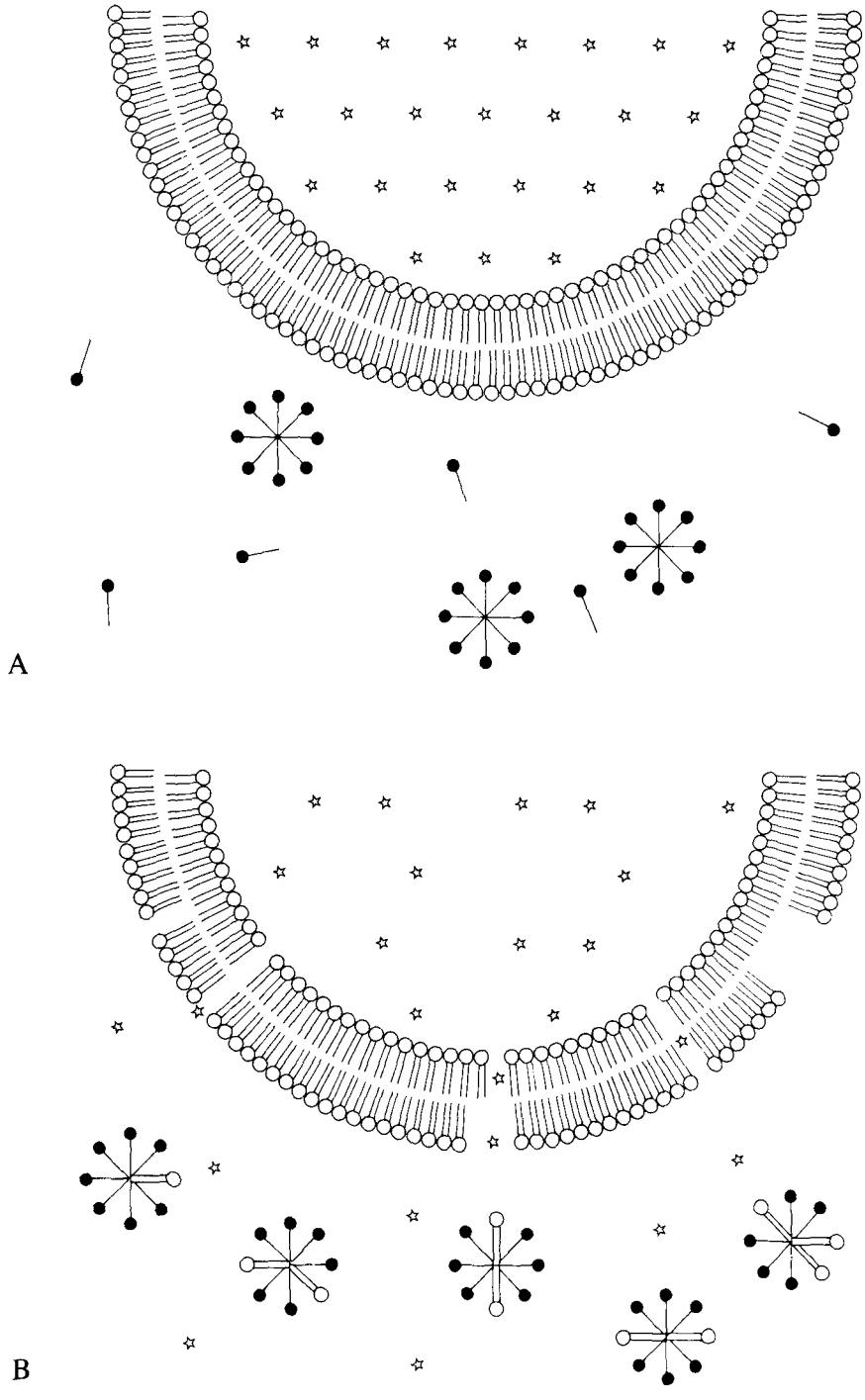

Fig. 1. Schematic presentation of the effect of detergents on liposomes. Phospholipids (◯=), peroxidase (☆~), and detergent (●__). (A) Before release of enzyme, the peroxidase is entrapped in liposomes and the detergent is either free or forming micelles. (B) After release of the enzyme, the phospholipids and detergent form mixed micelles and the peroxidase is liberated.

test tube. The current of nitrogen was continued for another 15 min. Then 250 μL of phosphate buffered saline (0.1 mol/L sodium phosphate, 0.15 mol/L sodium chloride), pH 7.5, (PBS) containing 2 mg of peroxidase (E. C No 1.11.1.7) was added to the tube. Liposomes were formed by vigorously vortexing the test tube for 5 min before dilution with 740 μL PBS.

Assay of Peroxidase Leakage from the Liposomes

Twenty microliters of liposome suspension was deposited on a paper disc (diameter 14 mm, Munktells filterpapper no 3). To remove any unbound peroxidase, the discs were washed four times for 5 min, each time in 4 mL of PBS. The filter paper was then sucked dry with a Pasteur pipet. One milliliter of PBS containing the de-

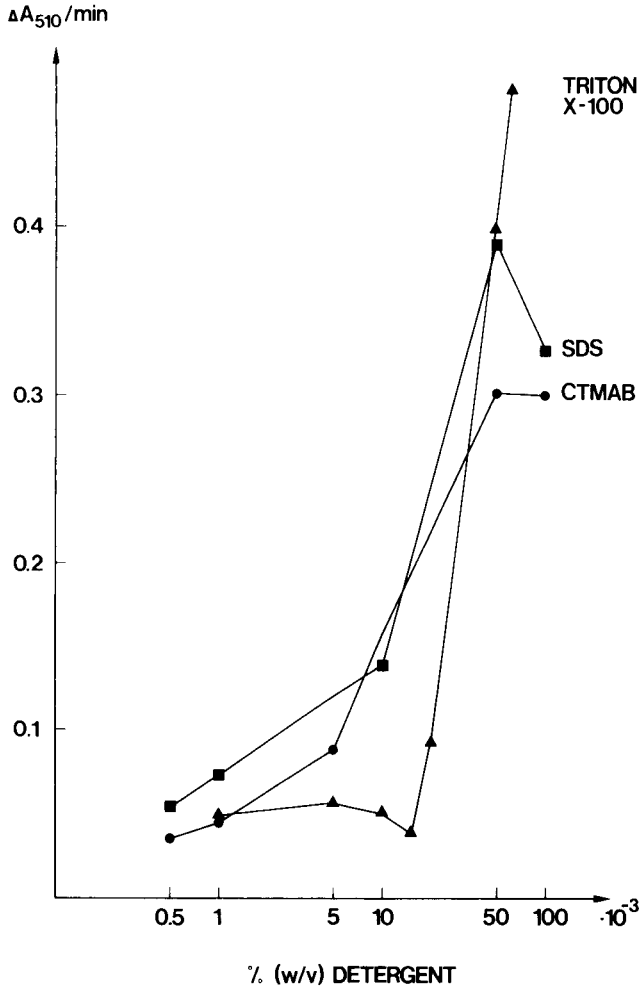

Fig. 2. Calibration curves for the non-ionic detergent Triton X-100 (▲), the ionic detergents sodium dodecyl sulfate; SDS (■); and cetyltrimethylammonium bromide (●). The experimental details are given in the text.

tergent was deposited on the paper disc, and the reaction vessel was covered and left for 4 h at room temperature. Half a milliliter of the incubation solution was then used for measuring the liberated peroxidase (*4*).

Results and Discussion

Calibration curves (Fig. 2) for the ability of detergents to destroy a membrane structure and thereby liberate enzyme can be constructed by this liposome method (*6*). The detergents vary somewhat in concentration necessary to solubilize the liposomes. Non-ionic detergents show an almost constant release of entrapped marker enzyme at concentrations below the CMC. At the CMC the effect decreases to a minimum and only at higher concentrations can a rapid release of marker enzyme by the detergent be seen (Fig. 2). The pattern of the calibration curves for anionic and cationic detergents is quite different. The CMCs of these detergents decreases with increasing counterion concentration (*7*), which makes it difficult to predict the exact CMC. The calibration curve shows a three-to-fivefold increase in the slope of the calibration curve in the concentration range of 0.005–0.01% (w/v) for the cetyltrimethylammonium bromide and 0.01–0.05% (w/v) for the SDS (Fig. 2). These observed concentration ranges of the CMCs of the detergents corresponds to those expected (*7, 8*). At higher concentrations of ionic detergents, a denaturating effect on the released enzyme is observed (Fig. 2).

This method for measuring detergents can be applied in concentration ranges where the detergents have their biological effect, and by comparing the calibration curves for different detergents a relative figure for the biological effect of each detergent can be obtained. Further development of the method would make it suitable as a quick and simple way to quantify the total membrane-destroying effect in a sample.

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