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ENHANCED ENTRAPMENT OF ISOPROPAMIDE IODIDE IN LIPOSOMES BY ION-PAIRING WITH SODIUM TAUR0DEOXYCHOLATE

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ABSTRACT

A study was carried out to determine the effect of sodium taurodeoxycholate (TDC) on the encapsulation efficiency of isopropamide iodide(IPM) in large unilamellar liposomes prepared by reverse phase evaporation method. The apparent partition coefficient of IPM between n-octanol and phosphate buffer(pH 7.4) was approximately zero, but it was increased by addition of TDC below CMC(1×10^{-2} M). The increased partitioning of IPM into n-octanol seemed be due to ion-pair complex formation between IPM and TDC. The encapsulation efficiency of IPM by

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the liposome, which was determined by equilibrium dialysis method, was increased from 21% to 62% by the presence of TDC(1×10^{-2} M). It may be due to the solubilization of the ion-pair in the phospholipid bilayer of the liposome.

INTRODUCTION

Liposomes are microscopic vesicles composed of membrane-like lipid layers surrounding aqueous compartments. In recent years, liposomes, phospholipid bilayer vesicles, are increasingly being utilized potential as drug carrying vesicles[1]. Liposomes have been administered intravenously and orally[2], and a recent report describes the delivery of drugs via liposomes by the topical route[3]. Their use allows the reduction of dosages, minimizes immunological and toxicological responses[4-7], increases cellular permeability and delays drug elimination. Equally importantly, liposomes are completly biodegradable and do not, therefore, elicit cytotoxicity. For these reasons, liposomes have been viewed as an attractive mechanism for drug delivery, especially for biologically unstable compounds, but not without certain drawbacks.

The very first requirement for the successful use of liposomes as drug carrier is to ensure the entrapment of sufficient amounts of the therapeutic agent. Jay et al[8]. used trichloroacetate to increase the entrapment of a quarternary ammonium compound in lipcsomes by an ion-pairing. By the way trichloroacetate is a caustic and so, the application of liposomes containing such a drug to biological systems is not appropriate. In this report, TDC, an endogenous bile ingredient, was examined to increase liposomal entrapment of IPM. This idea was stimulated by the report[9] that the ion-pair complex of IPM with TDC was more lipophilic than with bezoate, p-toluenesulfonate or salicylate.

MATERIALS AND METHODS

Materials

Isopropamide iodide(IPM, Yuhan Co Korea) and sodium taurodeoxycholate (TDC, Sigma Chem. Co., USA) were selected as cationic and anionic compound respectively. Egg yolk lecithin(Tokyo Kasei Kogyo Co.,Japan) was purified by alumina column chromatography and the purity was established by thin layer chromatography. Cholesterol(Nakarei

Chem. Co., Japan) was recrystallized several times from absolute ethanol. All the other chemicals were reagent grade and were used without further purification.

Quantitative Analysis of IPM

For the analysis of IPM, a modification of Santoro[10] was employed. A 2ml qaliquot of aqueous phosphate buffer(pH7.4) containing IPM was added to a screw-capped test tube containing 5ml of methyl orange buffer (pH10.2)[11] and 10ml of chloroform. After vigorous agitation with vortex mixer for 5min,two phase were separated by centrifugation at 3000 rpm for 5 min and aqueous phase was discarded by aspiration. A 2ml aliquot of 0.5N-HCl in absolute ethanol was added to 5ml of chloroform layer to develop the color. Then, the absorbance was measured spectrophometrically at 525nm.

Partition Studies

n-Octanol saturated with 0.1M-phosphate buffer(pH7.4) and 0.1M phosphate(pH7.4), saturated with n-octanol were used. IPM and TDC were dissolved in 0.1M-phosphate buffer (pH7.4) saturated with n-octanol. The concentration was kept constant at 1×10^{-4} M with only the concentration of

TDC being varied. A 10ml aliquot of each aqueous buffer solution containing IPM and TDC was mixed vigorously with 19ml of n-octanol in screw capped test tube for 5min and was shaken for 2 hr at 25 °C in a temperature-controlled water bath. After standing 30 min at 25 °C water bath ,two layers were seperated by centrifugation at 3000 rpm for 10 min and the n-octanol layer(upper layer) was discarded by aspiration. The buffer layer then was assayed for IPM. The concentration of IPM in the buffer before and after partitioning were measured and apparent partition coefficient(APC) of IPM was calculated by the difference. n-Octanol was chosen on the basis of its favorable non-polar lipoidal character[12].

Preparation of Liposomes

The lipid mixture (16.5 micromole phosphatidylcholine + 3 micromole cholesterol) dissolved in chloroform was added to a 35 ml round bottomed flask with long extention neck, and chloform was removed under reduced pressure by rotatory vacuum evaporator. The thin film formed was redissolved in 3 ml of diethyl ether, in which the reversed phase vesicles would be formed. 1 ml of 0.1M-phosphate buffer containing IPM of 1×10^{-4} M and varing concentrations of TDC was added at this

point and resulting two phase system was sonicated for 2 min in tip-type sonicater at 0-5 °C. The mixture was then subjected to rotary evaporator (2000 rpm) to remove ddiethyl ether at 20-25°C. As the majority of diethyl ether was removed, the material first formed a viscous gel and subsequently it became an aqueous suspension. At this time excess aqueous phase could be added (but not necessary) and the suspension was evaporated additionally to remove traces of diethyl ether. Formation of liposomes was confirmed by microscopy(x1500).

Gel Filtration of Liposomes

2ml aliquots of liposomes were applied to a column of Sepharose 4B. The column had a diameter of 2.3cm and length of 60cm. 0.1M-phosphate buffer(pH7.4) containing 0.02% sodium azide as preservative was used as eluent. Flow rate was 24ml/hr and 4ml fractions were collected and 3 ml aliquots were taken from each tube for assay. The turbidities of aliquots were determined spectrophotometrically at 300 nm.

Determination of Entrapment Efficiency

Entrapment efficiiency was determined by equilibrium dialysis method. 3ml aliquots of

liposomes and 0.1M-phosphate buffer (pH 7.4) were put into each side of the membrane (Visking Co., type 18/32, U.S.A.) of the dialysis cell (Natsume, Japan) respectively. The cell was shaken for 24 hr at 4 ℃ in the water bath. Then the concentration of free IPM in the buffer was determined. Since the concentration of IPM was 1×10^{-4} M originally, following equation could be used for the calculation of the entrapment efficiency:

$$\text{Entrapment Efficiency}(\%) = (1\times10^{-4} - 2\times[\text{IPM}])/(1\times10^{-4}) \times 100$$

where [IPM] means the molar concentration of IPM in buffer side of the membrane. The dialysis membrane used in this study was pretreated as follows; It was swollen sufficiently in distilled water. After heating for 1 hr in 1 liter of 50% ethanol solution, it was cooled to room temperature. Again, it was washed with 1 liter of 0.01M-NaHCO3 solution for 2hr and then recooled to room temperature. After heating for 2 hr in 1 liter of EDTA solution, it was kept in 0.02%-sodium azide solution at 4 ℃.

RESULT AND DISCUSSION

Effect of TDC on APC of IPM

Irwin et al[13] defined ion-pairs as neutral species formed by electrostatic attraction between

oppositely charged ions in solution, which are often sufficiently liphophlic to dissolve in non-aqueous solvents. Ions that are unite in some fashion to form ion-pairs essentially cancel all or part of the charge of each ion, and thus there are fewer species in the solution having an exposed charge. The partitioning of chareged species into non-aqueous medium results from the masking of hydrophlic site by counterion. Some counterions are more effective than others in increasing the lipophilicity of the charged species. The use of ion pairing techniques has been utilized to enhance the lipid solubility of certain ionic compounds. As was shown in Fig.1 TDC progressively increased the partitioning of IPM from a aqueous buffer into n-octanol, which indicates possible formation of lipid soluble ion-pair complex, but increased partitioning was inhibited by TDC above 1×10^{-4} M, which was reported as CMC of TDC. IPM didn't partition into n-octanol in the absence of TDC (Fig.1).

Microscopy and Gel Filtration of Liposome

Liposomes were bounded by a single bilayer and had an average diameter of 200 A°. They were alike one another externally regardless of concentration of TDC added below CMC. The detergent effect bile

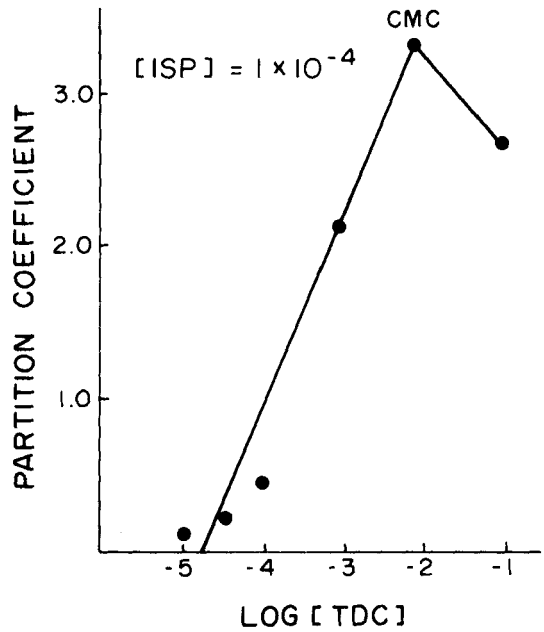

FIGURE 1

Apparent partition coefficients (APC) of ISP between n-octanol and 0.1M-phosphate buffer (pH7.4) in the presence of various concentrations of TDC.

salts on liposomal membrane[14] did not appeared at concentrations below CMC of TDC. The elution profile of the liposomes containing IPM and TDC through Sepharose 4B column was similar to that of the liposomes containing IPM only (Fig.2).

Effect of TDC on Liposomal Entrapment of IPM

To utilize liposomes as drug carrier, one major problem still exist, i.e., many drugs, especially

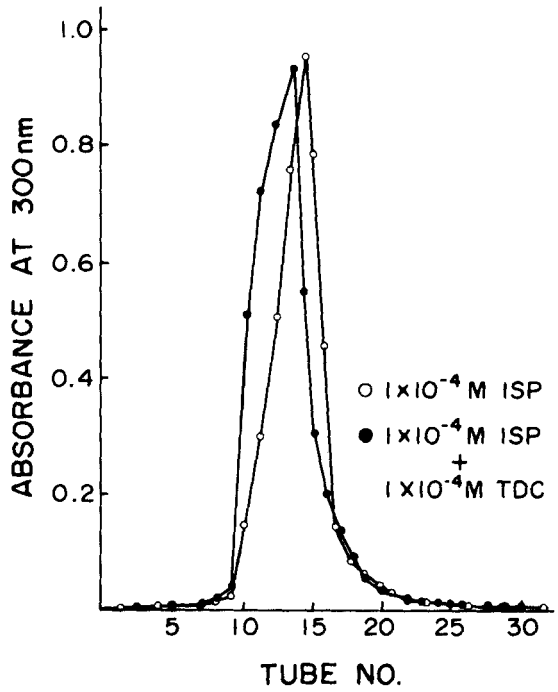

FIGURE 2

Gel filtration of liposomes on Sepharose 4B column. Liposome was prepared with $1x10^{-4}$M ISP only (O),or 1×10^{-4}M ISP plus 1×10^{-4}M TDC (O).

water-soluble drugs, are poorly encapsulated by liposomes. For reference, the reported efficiencies of drug encapsulation into liposomes range from 0.1% to 10% per micromole phospholipid [15], depending on the nature of the drug and of liposome employed. In this study, the entrapment of IPM by large unilamellar liposome was enhanced about 3-fold, from

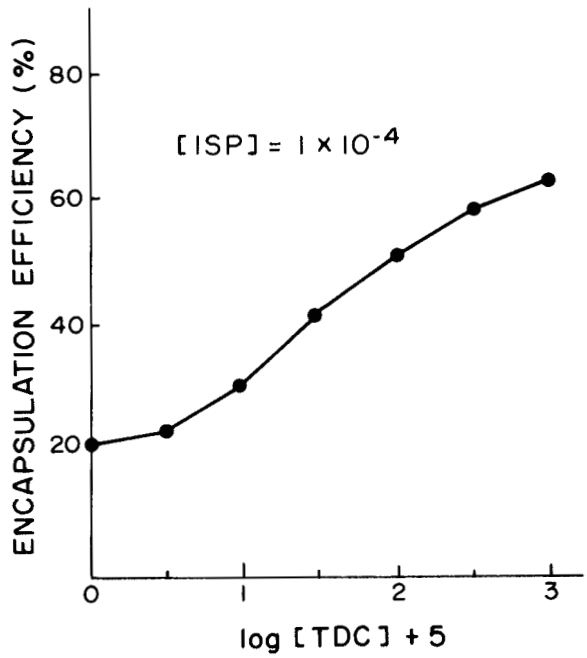

FIGURE 3

Change of encapsulation efficiency (%) of ISP in liposome in the presence of various concentrations of TDC.

21% at control to 62% in the presence of 1×10^{-2} M of TDC(Fig.3). The increased liposomal entrapment of IPM was probably due to the solubilization of the ion-pair, IPM-TDC, in the lipid membrane of the liposome.[8] This ion-pairing technique seems to be useful to enhance the entrapment efficiency of many polar drugs in liposomes.

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