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Length of hydrocarbon chain influences location of curcumin in liposomes: Curcumin as a molecular probe to study ethanol induced interdigitation of liposomes

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ABSTRACT

Using fluorescence quenching of curcumin in 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) liposomes by brominated derivatives of fatty acids, the location of curcumin has been studied, which indicates length of hydrocarbon chain has an effect on the location of curcumin in liposomes. Change of fluorescence intensity of curcumin with temperature in the presence of liposomes helps to estimate the phase transition temperature of these liposomes, thus, influence of cholesterol on liposome properties has been studied using curcumin as a molecule probe. The cooperativity due to the interactions between the hydrocarbon chains during melting accelerates the phase transition of DPPC liposomes in the presence of high percentage of cholesterol whereas high percentage of cholesterol generates a rather rigid DMPC liposome over a wide range of temperatures. We used ethanol to induce interdigitation between the hydrophobic chains of the lipids and studied this effect using curcumin as fluorescence probe. As a result of interdigitation, curcumin fluorescence is quenched in liposomes. The compact arrangement of the acyl chains prevents curcumin from penetrating deep near the midplane. In the liquid crystalline phase ethanol introduces a kind of order to the more fluid liposome, and does not leave space for curcumin to be inserted away from water.

1. Introduction

1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione, commonly known as curcumin is a yellow pigment found in the rhizomes of *Curcuma longa* (turmeric). Besides its wide application as food spices, especially in South Asia since ancient time, recently focus on this molecule has been tremendously rising for its pharmaceutical applications (including for anti-cancer, anti-amyloid, anti-inflammatory and anti-oxidant activities) [1–7]. Curcumin has long been used in Asian medicine for its healing and preventive properties. Demand for curcumin as a supplement has grown extremely in the past five years. In addition, the safe profile of curcumin has generated curiosity among researchers, with no toxicity to healthy organs at doses as high as 8 g day^{-1} in clinical trials [8]. It is soluble in nonpolar organic solvents; it is also soluble in slightly alkaline aqueous solutions and solutions containing micelles [9]. Therefore, its interaction with membrane/liposomes has been of great interest.

The absorbance of curcumin is little affected by the nature of the solvent; a red shift is observed when the polarity of the solvent is increased. The peaks are broad and reveal several shoulders indicating the presence of different isomers [10]. In fact in its enol form, curcumin can exist as cis and trans isomers that lead to observed absorption spectra. On the other hand, the fluorescence intensity and the position of the maxima depend on the nature of the solvent. In nonpolar solvents the spectra can be well resolved into Gaussian bands, for example, while in more polar solvents the spectra are broad. A large red shift is observed when the solvent becomes more polar: a maximum of 446 nm in cyclohexane to 549 nm in dimethylsulfoxide. But the position of the maxima not only varies with the polarity but also with the ability of the solvent to create hydrogen bonding [10]. The different cis–trans isomers of the enol show no dependence on the excitation wavelength, this indicates that either all isomers yield the same fluorescent excited state or that they all behave similarly in steady-state fluorescence [9]. Due to its exciting fluorescence properties and safe profile, recently we have been studying this molecule for potential spectroscopic application in molecular probe [11–14] and sensing application [15,16].

In spectroscopy the use of fluorescence for probing liposomes has many advantages over classical microscopy techniques since it provides information about minute details reaching single molecule sensitivity, and permits the study of the dynamics of lipids and proteins on narrow timescales. Probing can be either direct, by visualizing a fluorescent component of the liposome such as green fluorescent proteins, or by inserting fluorescent compounds or dyes into the liposome. The only problem is the significant size of the probes relative to the small

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phospholipid molecules [17]. An alternative to direct labeling is adding the probe in vitro to lipids such as cholesterol, sphingomyelin, phosphatidylcholine or phosphatidylethanolamine, and then incorporating them into the liposome by fusion. Moreover some lipophilic fluorescence probes penetrate into the liposome, thus they reveal some of the properties of the bilayer such as the order, the pH, and the viscosity [17–21]. Identifying new fluorescence probe or marker to investigate liposome properties has been a topic of great interest among spectroscopists [17–21]. We have established successfully that curcumin can act as an efficient liposome probe to investigate fluidity and phase transition temperature. The partition coefficient of curcumin with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-Dipalmitoyl-sn-Glycero-3-Phosphocholine (DPPC) liposomes was measured and found to be high. We concluded that curcumin binds strongly to liposomes and at high concentrations curcumin influences the phase transition temperature of liposomes but at low concentration it does not influence it appreciably [12,13]. In this paper, we have identified location of curcumin in liposomes to understand its interaction and investigated curcumin as a probe molecule to examine effect of cholesterol on liposomes and study ethanol induced interdigitation of liposomes by applying curcumin as a spectroscopic probe molecule. The results obtained open a new direction to the use curcumin for studying various other liposome properties and understand its interaction with liposome's interfaces.

2. Materials and Methods

2.1. Materials

DPPC, DMPC, 2-bromohexadecanoic acid, 11-bromoundecanoic acid and 16-bromohexadecanoic acid were obtained from Sigma-Aldrich and used as received. Curcumin was obtained from Acros Organics and was used without further purification. The solvents used are of spectroscopic grade and obtained from Acros Organics or Sigma-Aldrich.

2.2. Methods

The stock solution of liposome was prepared in 50 mM mono and dibasic phosphate buffer at pH 7.0. Further dilution was made in 50 mM mono and dibasic phosphate buffer at pH 7.0. As curcumin is poorly soluble in water and degrades in aqueous environment, the stock solution of curcumin was made in spectroscopic grade methanol. Few μL of the stock sample of curcumin (~mM) was taken in a vial and added to final sample for measurement in buffer. Curcumin in liposome solution was fairly stable for pH value under study. It was made sure that the final concentration of methanol is negligible, <0.1% in the measurement sample and does not affect the sample.

2.3. Preparation of Liposomes

The desired amount of phospholipid was dissolved in 15 mL of a mixture of chloroform-methanol at 2:1 volume ratio. The solvents were evaporated using a rotary evaporator at 57 to 60 °C. The liposome formed was dried under vacuum for 10 min. Then glass beads were added, and enough phosphate buffer at pH 7.0 was used to form liposomes at a concentration of 1 mM. The mixture was vortexed rigorously for 15 min and finally heated for 30 min at 52 °C for DPPC, and 37 °C for DMPC (above the phase transition temperatures). The preparation technique of liposomes was modified for other experiments. For instance, the interdigitation with ethanol and the quenching studies were done with liposomes prepared by dissolving the phospholipids in 2 mL of chloroform–methanol in a 1:1 volume ratio, then evaporating the solvents using a rotary evaporator at 60 °C. Enough mono and dibasic phosphate buffer at pH 7.0, and at 37 °C for DMPC, and 50 °C for DPPC, was added to obtain a final concentration of 10 mM. Finally the mixture was rigorously vortexed for 5 min at a constant temperature (37 °C and 50 °C respectively for DMPC and DPPC). The concentration of liposomes was reported based on lipid concentration in the medium.

2.4. Incorporation of Additives

Incorporation of quencher: The long chain brominated quencher, 2-bromohexadecanoic acid, was mixed with DPPC or DMPC lipid during liposome preparation with a molar ratio [lipid]:[quencher] = 85:15 in chloroform–methanol (2:1) solvent; the solvent was evaporated using a rotary evaporator above the phase transition temperature and liposomes preparation was continued as described above. Similar procedure was separately repeated for 11-bromoundecanoic acid and 16-bromohexadecanoic acid using DMPC as well as DPPC.

Incorporation of cholesterol: Cholesterol was added to the liposome during the synthesis process. A stock solution of curcumin 1 mM was prepared in methanol, and the desired amounts were added to the phospholipid's mixture with the chloroform–methanol at the early stages of the preparation of the liposomes. The remainder of the procedure was resumed as described earlier.

Ethanol-induced interdigitation: The desired amounts of ethanol were added to the liposome solutions containing curcumin. 3 mL samples were obtained by completing the volume with mono and dibase phosphate buffer at pH 7.0.

2.5. Spectroscopic Measurements

The absorption spectra were recorded at room temperature using a JASCO V-570 UV–VIS–NIR Spectrophotometer. The steady-state fluorescence (emission and excitation) measurements were also recorded with resolution increment 1 nm, slit 5 using a Jobin-Yvon-Horiba Fluorolog III fluorometer and the FluorEssence program. The excitation source was a 100 W Xenon lamp, and the detector used was R-928 operating at a voltage of 950 V. To regulate the temperature a thermostat was coupled with the sample holder and the final temperature was noted in the sample compartment using a thermometer rather than a thermostat.

2.6. Fluorescence Lifetime Measurements

The fluorescence lifetime measurements were done using a Jobin-Yvon-Horiba Fluorolog III fluorometer, with a pulsed diode laser of 405 nm. The decay data was analyzed using Data Analysis Software. The fluorescence decay was acquired with a peak preset of 10,000 counts. The detector used was R-928 operating at a voltage of 950 V. The instrumental response function was collected using a scatterer (colloidal silica). The best fit was with a χ^2 value in the range 0.9 to 1.5.

3. Results and Discussion

3.1. Interaction of Curcumin with Liposomes

Curcumin incorporates into DPPC and DMPC liposomes due to the fact that it has a high affinity towards lipids. Hence the absorbance increased proportionally to the amount of liposome in the sample (see Fig. 1A&B). The fluorescence spectra of curcumin in both liposomes revealed a significant blue shift (ca 55 nm) as compared to that in buffer solution. The maxima shifted from 550 nm in water to 495 nm in both liposomes. This indicates that curcumin is partitioning into the vesicle. Representative fluorescence spectra and intensity change of curcumin upon incorporation with various concentrations of DMPC and DPPC are given in Fig. 1C&D. The data clearly suggest a strong fluorescence of curcumin in liposomes. The fluorescence lifetime of curcumin in the DMPC and DPPC liposomes was found to be 674 ps (with a 5.16 ns long component) and 427 ps (with a 2.55 ns long component), respectively, which is similar to that in non-polar solvent and less than that in the buffer solution confirming incorporation of curcumin into the