J Incl Phenom Macrocycl Chem (2007) 57:309–311
DOI 10.1007/s10847-006-9178-y

ORIGINAL ARTICLE

Study of the interaction between cyclodextrins and liposome membranes: effect on the permeability of liposomes

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Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 13 February 2007

Abstract We wanted to compare and understand the effect of the most currently used cyclodextrins on a model membrane. We studied the influence of most currently used cyclodextrins on the release of a fluorescent marker encapsulated in the inner cavity of SUV liposomes. It was shown that the observed effect on calcein release can be directly related to the affinity of cyclodextrins for both lipid components of liposomes, cholesterol and phosphatidylcholine. From this relationship, we were able to determine, for each cyclodextrin, a theoretical concentration giving rise to 50% or 100% calcein release. This theoretical concentration was confirmed experimentally.

Keywords Liposome · Cyclodextrin · Cholesterol · Calcein · Membrane permeability

Introduction

It is well known that cyclodextrins are able to extract lipids constituting cell membranes, increasing their fluidity and permeability [1–4]. This behaviour towards biological membranes is directly linked to the toxicological effects of methylated cyclodextrins. We wanted to compare and understand the effect of the most currently used cyclodextrins on a model membrane. A detailed knowledge of the extraction of cholesterol and other lipid components by cyclodextrins can help us to better understand and predict what will happen when cell membranes are exposed to cyclodextrins.

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Material and methods

Soybean phosphatidylcholine (SPC), cholesterol (CHOL), stearylamine (SA), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and calcein were purchased from Sigma-Aldrich (Bornem, Belgium). β-cyclodextrin (βCD), hydroxypropylated β-cyclodextrin (HPβCD; D.S. 0.63) and Kleptose® Crysmeb (Crysmeb) were kindly donated by Roquette Frères (Lestrem, France). γ-cyclodextrin (γCD), randomly methylated β-cyclodextrin (Rameb), HPβCD (D.S. 0.8) and hydroxypropylated γ-cyclodextrin (HPγCD) were a gift from Wacker-Chemie GmbH (Munich, Germany). 2, 6 dimethyl- β-cyclodextrin (Dimeb), 2, 3, 6 trimethyl- β-cyclodextrin (Trimeb) and HPβCD (D.S. 0.4) were obtained from Cyclolab (Budapest, Hungary). Sulfobutylether-β-cyclodextrin (SBEβCD) came from Cydex (Kansas, USA).

We studied the influence of βCD, γCD, Dimeb, Trimeb, Rameb, Crysmeb, HPβCD (of different substitution degrees), HPγCD and SBEβCD on the release of calcein, a fluorescent marker encapsulated in the inner cavity of small unilamellar liposomes. SUV made from SPC:CHOL:SA (60:30:10 mol%) or SPC:SA (90:10 mol%) were prepared by hydration of lipid films. In practice, all runs were initiated by adding

100 μl of liposome suspension (adjusted to a 0.15 mM SPC concentration) to 100 μl of a buffered solution (pH 7.4) of cyclodextrin (0–100 mM) or a HEPES buffered solution (blank test) or a 2% Triton X-100 solution, which had been previously placed in a 96-well plate and heated at 37°C. The release of calcein from the liposomes was measured fluorometrically immediately after the addition of the liposome suspension and after 2.5, 5.0, 7.5, 10.0, 15.0, 20.0 and 30.0 min. The amount of calcein released was calculated by the following equation: % calcein released = $(I_t\text{-}I_0)/(I_T\text{-}I_0)$ where I_t is the fluorescence intensity at 520 nm at time t after the addition of the liposome suspension, I_0 is the fluorescence intensity of calcein loaded liposome suspension in a buffered solution without cyclodextrin, and I_T is the fluorescence intensity after complete destruction of the liposomes by triton X-100.

Results and discussion

Dimeb at a concentration higher than 20 mM, induced a total calcein leakage immediately after its addition. With the addition of βCD, Crysmeb, HPβCD (whatever the substitution degree), HPγCD and SBEβCD, no significant increase of the calcein leakage was observed. In the presence of γCD, Rameb and Trimeb, calcein was released at a level dependent on the cyclodextrin concentration. After the same period of time and at the same cyclodextrin concentration, the amount of calcein released increases in the order Rameb > Trimeb > γCD.

When membranes are enriched in cholesterol, liposomes are more sensitive to methylated cyclodextrins. For cyclodextrins, the presence of cholesterol has the opposite effect than that observed for conventional detergents [5]. This may be explained by the high affinity of these cyclodextrins for cholesterol. The effect of cyclodextrins on liposomes can be classified as follows: Dimeb > Rameb > Trimeb > γCD > βCD = HPβCD (whatever its substitution degree) = HPγCD = SBEβCD = Crysmeb. This indicates that methylated cyclodextrins, apart from Crysmeb, interact with lipid molecules and, as a consequence, the small calcein molecules escape from the liposome vesicles through pores (or else disruptions of the membrane continuity), which are formed on the membranes during lipid removal.

In order to explain the results obtained with calcein and, since soluble complex formation between cholesterol and cyclodextrin is strongly suggested, we therefore investigated quantitatively the interaction of cholesterol with these cyclodextrins in aqueous solution and related these results to those of calcein leakage.

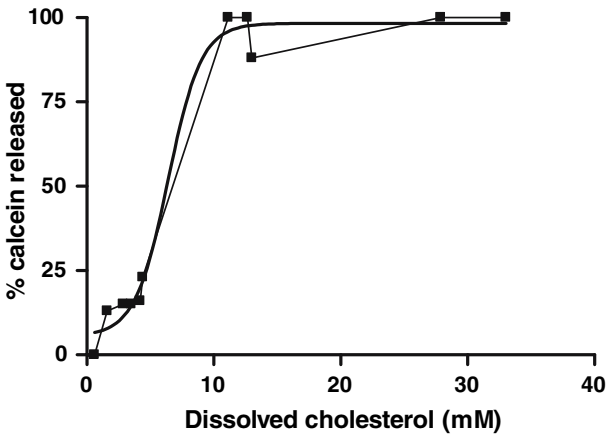

Fig. 1 Correlation between the percentage of calcein released from SPC:CHOL:SA liposomes after 30 min of contact with a determined concentration of cyclodextrin* and the quantity of cholesterol (mM) dissolved by the same quantity of the same cyclodextrin**. *results obtained from calcein release study; **results obtained from phase solubility diagram

Results show that the affinity of cyclodextrins for cholesterol increases in the order βCD = γCD = HPγCD = SBEβCD < HPβCD < Crysmeb < Dimeb < Rameb = Trimeb. Concerning SPC, the order of affinity was βCD = γCD = HPγCD = SBEβCD < HPβCD < Crysmeb < Trimeb < Rameb < Dimeb but complexation efficiencies were systematically lower than for cholesterol. It was shown in Figure 1, that the observed effect on calcein release can be directly related to the affinity of cyclodextrins for both lipid components of liposomes, cholesterol and phosphatidylcholine. Excluding the point corresponding to Dimeb 20 mM, correlation of the results follows a sigmoidal relationship, with an r^2 value of 0.9885. From this relationship, we were able to determine, for each cyclodextrin, a theoretical concentration giving rise to 50 or 100% calcein release. This theoretical concentration was than confirmed experimentally. Rameb and Trimeb confirmed our theoretical results, since these cyclodextrins do not provoke calcein leakage at a concentration lower than 20 mM. On the other hand, Dimeb provoked complete calcein release even at 10 mM as well as a partial release at 5 mM. This may be explained by the fact that Dimeb has also a strong affinity for SPC. This is confirmed by the fact that at 10 mM, Dimeb provokes a complete leakage of calcein from liposomes without cholesterol.

We also showed that calcein release is due to large structure modifications. PCS analyses showed that Rameb, Trimeb and Dimeb at 50 mM completely modify the size and probably the structure of liposomes, while Crysmeb at the same concentration does not.

Conclusion

This study has shown that the effect of cyclodextrins on calcein release can be directly related to the affinity of these cyclodextrins to the lipid components of liposomes. The effect of cyclodextrins on calcein release from liposomes can be classified as follows: Dimeb > Rameb > Trimeb > γCD > βCD = HPβCD (whatever its substitution degree) = HPγCD = SBEβCD = Crysmeb. Compared to other methylated derivatives, the low substitution degree of Crysmeb decreases its affinity for cholesterol and phosphatidylcholine. However, Crysmeb has good solubilizing properties for other substances. The selective solubilizing properties of this derivative for active substances instead of lipid components may be an interesting property, since cyclodextrin behaviour towards biological membranes, and especially their haemolytic and cytotoxic effects, are widely accepted to be related to lipid complexation and depletion.

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