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Effect of penetration enhancers on the phase transition of multilamellar liposomes of dipalmitoylphosphatidylcholine. A study by differential scanning calorimetry

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Summary

Multilamellar liposomes of dipalmitoylphosphatidylcholine were used as a simple model of lipid bilayers representing the intercellular lipid arrangement of the stratum corneum. The effect of skin penetration enhancers such as 1-dodecylazacycloheptan-2-one (Azone), oleic acid (OA), dimethyl sulphoxide (DMSO), thiamorpholine-3-one and *N*-dodecylthiamorpholine-3-one (C12-thiamorpholine-3-one) on the liposomal bilayer was assessed using differential scanning calorimetry. Azone, OA and C12-thiamorpholine-3-one dramatically modified the thermotropic behaviour of the phospholipid. The pretransition peak vanished at low penetration enhancer concentrations and an increase in the penetration enhancer:lipid molar ratio broadened the gel-to-liquid crystalline transition peak and shifted it to lower temperatures. For the tested concentrations, neither DMSO nor thiamorpholine-3-one changed the phase transition temperature of the liposomes.

Introduction

In recent years, the role of the stratum corneum in the barrier function of the skin has been thoroughly investigated (Scheulplein, 1976; Schaefer et al., 1982; Barry, 1983; Cooper and Berner, 1985; Moore et al., 1988; Wertz and Downing, 1989). Two major routes of drug penetration through the human stratum corneum have been proposed: the transcellular and the intercellular pathways (Michaels et al., 1975; Elias, 1983; Barry, 1987). For most drugs, the major barrier effect of the stratum corneum has been attributed to the intercellular lipids, mainly to their nature and to their ordered multilayers (Gray and White, 1979; Elias, 1981; Grayson and Elias, 1982; Lampe et al., 1983; Wertz et al., 1986; Madison et al., 1987; Williams and Elias, 1987; Wertz and Downing, 1989). Consequently, in order to extend the variety of drugs that might be administered via the skin and also to increase the local activity of topically applied drugs, considerable attention

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has recently been focused on the mechanism of action of skin penetration enhancers (Stoughton, 1982; Aungst et al., 1986; Barry, 1987; Walters, 1989).

Most of the penetration enhancers have been proved to interact in some way with the stratum corneum lipid structure, generally by increasing the fluidity of the intercellular lipid bilayers (Barry, 1988). In order to understand better the mechanism of action of various classes of penetration enhancers, multilamellar vesicles (MLV or liposomes) of dipalmitoylphosphatidylcholine (DPPC) have been used as a simple model representing the lipid bilayers of the stratum corneum. Over the past few years, several physical techniques, such as nuclear magnetic resonance (NMR), electron spin resonance, X-ray diffraction, Raman spectroscopy and Fourier transform infrared (FTIR) spectroscopy (Knutson et al., 1985; Golden et al., 1986; Beastall et al., 1988; Gay et al., 1989; Potts, 1989) have been used to study the thermotropic behaviour of the lipids. Since thermodynamic parameters for the gel-to-liquid crystalline phase transition of liposomes are best obtained by differential scanning calorimetry (DSC), the effect of various potential penetration enhancers such as Azone, dimethyl sulphoxide, oleic acid and *N*-alkylthiamorpholine-3-ones, on the phase transition temperature of MLV was investigated using this technique.

Materials and Methods

Materials

Dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma (France) and used without further purification. 1-Dodecylazacycloheptan-2-one (Azone) was obtained from Nelson Research (U.S.A.); *N*-dodecylthiamorpholine-3-one (C12-thiamorpholine-3-one) and thiamorpholine-3-one were synthesized by Dr J. Maignan from L'Oréal (France). Dimethyl sulphoxide (DMSO) was purchased from Merck (France) and oleic acid (OA) from Sigma. The chemical structures of the potential penetration enhancers used in the study are presented in Fig. 1.

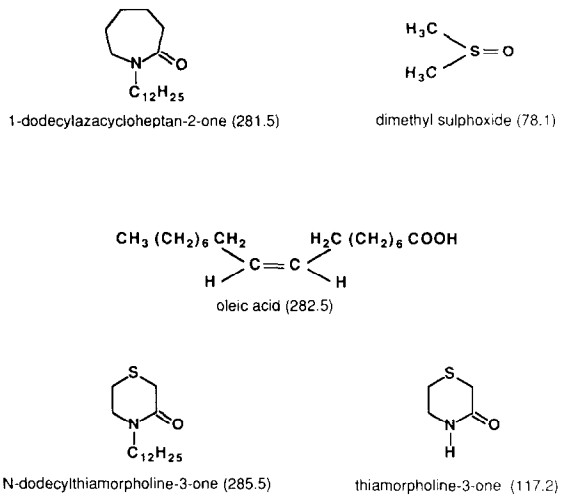

Fig. 1. Chemical structure and molecular weight of tested compounds.

Liposome preparation

Neutral liposomes (MLV) were prepared from DPPC using a procedure derived from the solvent evaporation method originally described by Bangham et al. (1974). Briefly, phospholipids dissolved in chloroform were dried under nitrogen to a thin lipid film deposited on the wall of a glass vessel. After overnight drying under vacuum, the lipid was resuspended in sterile water at 50 °C (temperature above the transition temperature of DPPC, 41.4 °C) by vigorous vortexing for 3 min every 10 min for 2 h. The suspension (20–50 mg/ml) was then left overnight at 4 °C in order to achieve maximal liposome hydration. Penetration enhancers (from 2.5 to 25 mol%) were incorporated in the liposome structure by first dissolving phospholipids and enhancers together in chloroform. The preparation of liposomes was then carried out as described above.

The formation of liposomes was controlled by polarised light microscopy and their diameter, size range and lamellarity by electron microscopy (Fig. 2).

Differential scanning calorimetry (DSC)

DSC was performed on a Perkin Elmer DSC 7 coupled to a Perkin Elmer 7700 computer. 15 μl of the liposomal suspension containing an average of 0.6 mg of DPPC were sealed in a 20 μl

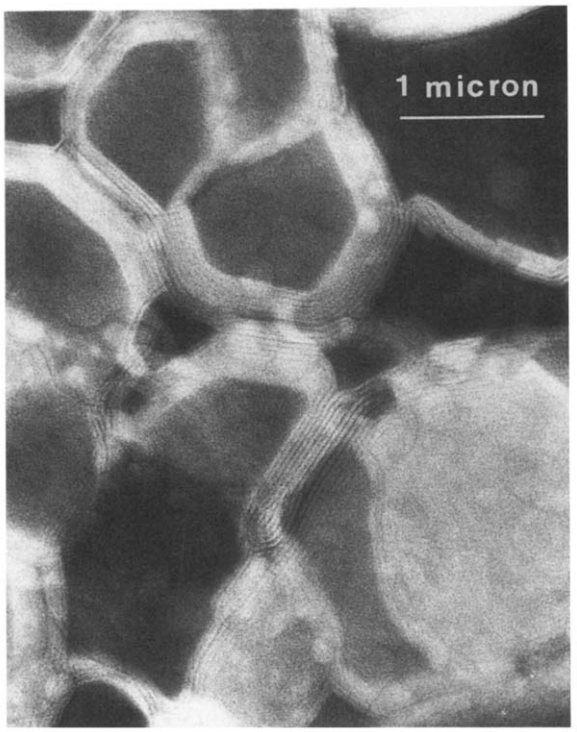

Fig. 2. Multilamellar liposomes of dipalmitoylphosphatidylcholine observed by transmission electron microscopy after negative staining with 2% phosphotungstic acid.

aluminium pan. The samples were analysed by heating at a scanning rate of 2 °C/min over the temperature range 20–50 °C, with a sensitivity of 4 mW full-scale, using sterile water as reference. The system was calibrated in temperature and heat flow using lauric and capric acid.

Results and Discussion

1-Dodecylazacycloheptan-2-one (Azone)

As observed in Fig. 3, for pure DPPC liposomes the excess specific heat reaches a maximum at 35 °C for the pretransition and at 41.5 °C for the main transition, which is in agreement with literature values (Mabrey-Gaud, 1981; Constantinides et al., 1986). These two endothermic transitions have been generally attributed firstly to rotations of the phospholipid head group or transformation in the lamellar structure and changes in hydrocarbon chain packing (pretransition temperature) and secondly to chain-melting transition (phase transition temperature) (Janiak et al., 1979; Mabrey-Gaud, 1981; Gruner, 1987).

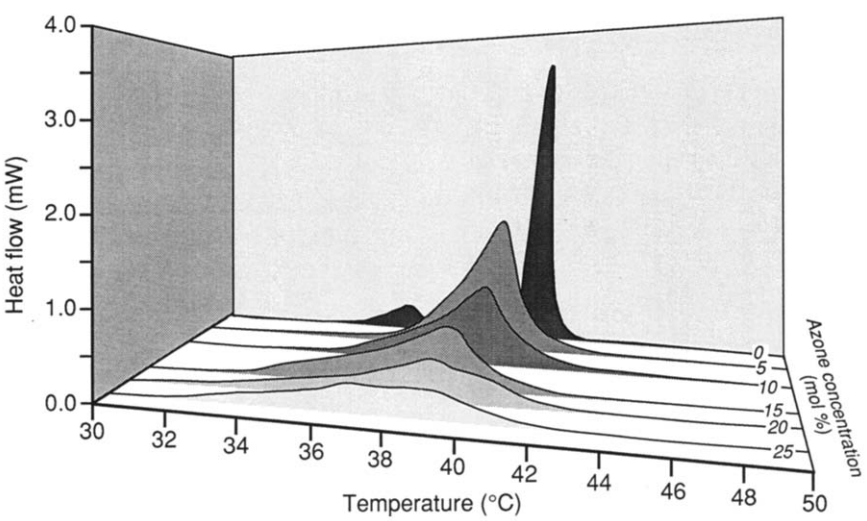

Fig. 3. DSC thermograms of DPPC liposomes containing increasing concentrations of Azone.

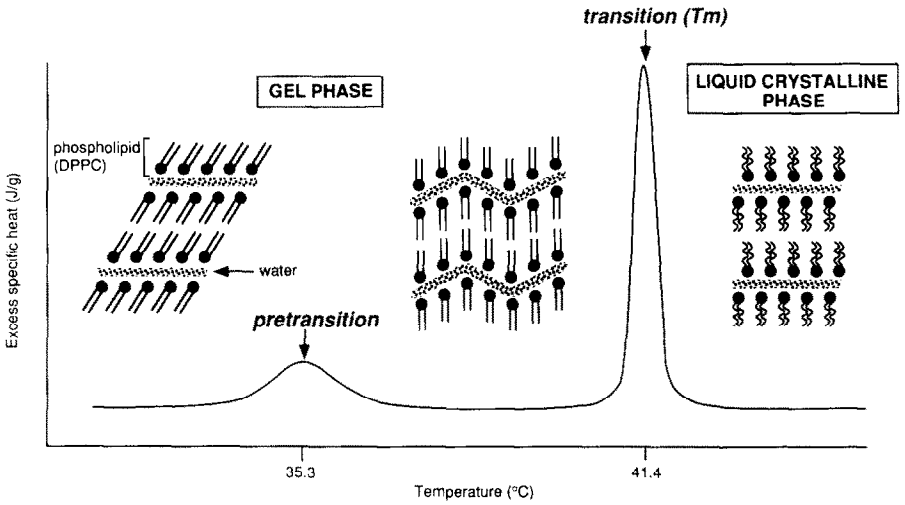

Fig. 4. Typical DSC curve illustrating the phase transitions of DPPC liposomes and the corresponding phospholipid bilayer structure.

At the phase transition temperature (T_m), liposomes undergo distinct structural changes, as illustrated in Fig. 4. Below T_m, lipids in the bilayers are in a highly ordered gel state, the hydrocarbon chains being in an all-*trans* configuration. At the pretransition temperature, lipids change from tilted one-dimensional arrangements to two-dimensional arrangements with periodic undulations (Fig. 4). Above T_m, the lipids become more fluid as a consequence of a *trans-gauche* rotational isomerisation along the chains resulting in a lateral expansion and decrease in thickness of the bilayer, and revert to one-dimensional arrangements (Mabrey-Gaud, 1981; Fendler, 1982). This so-called gel-to-liquid crystalline phase transition of multilamellar liposomes is specific of the phospholipid structure and occurs for DPPC liposomes at 41.4 °C (Figs 3 and 4).

Typical DSC thermograms of liposomes of DPPC, containing Azone at increasing enhancer: phospholipid molar ratios, are shown in Fig. 3. Increasing the Azone: DPPC molar ratios leads to a dramatic effect on the thermotropic behaviour of the phospholipid. Thus, the pretransition vanishes at low Azone concentrations (5 mol%) and the increase in Azone: lipid molar ratios broadens the gel-to-liquid crystalline transition peak and shifts it to lower temperatures.

The influence of Azone concentration on the phase transition of DPPC is shown in Fig. 5. T_m decreases as a function of Azone concentrations and the broadening of the phase transition is illustrated by an increase of $\Delta T_{1/2}$, which is the width at half-height of the main DSC peak.

Using another method, namely by measuring the turbidity of a liposomal suspension as a function of temperature, Beastall et al. (1988) similarly found that increasing Azone concentration decreased the onset temperature, broadened the transition and abolished the pretransition.

In a preliminary study, interaction of Azone with bilayers produced by aqueous polar surfactant systems was also investigated by small-angle

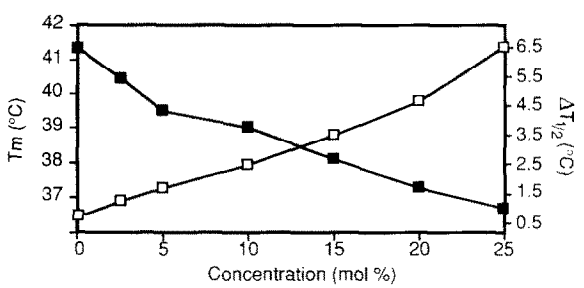

Fig. 5. Effect of Azone concentration on the phase transition temperature (T_m, ■) of DPPC liposomes and on the width at half-height of the main DSC peak ($\Delta T_{1/2}$, □).

X-ray diffraction and deuterium NMR measurements (Ward and Tallon, 1988). Azone was shown to increase the ability of the surfactant bilayer to incorporate water, thus probably facilitating penetration of hydrophilic drug molecules. In addition, the location of Azone molecules in the bilayer structure might potentially enhance absorption of hydrophobic molecules. The mechanism of action of Azone has also been extensively investigated in human skin by various authors (Goodman and Barry, 1985; Barry, 1987; Boddé et al., 1989; Bouwstra et al., 1989); using different techniques, e.g., DSC, it was suggested that this particular enhancer had a marked influence on the stratum corneum intercellular lipid fluidity and structure.

DSC of DPPC multilamellar liposomes might be therefore a predictive in vitro test for understanding properties of particular penetration enhancers, i.e., enhancers that interact with stratum corneum lipids.

Oleic acid (OA)

Oleic acid gave results very similar to those obtained with Azone. As a consequence of an increase in the OA:DPPC molar ratio, the pretransition vanishes at relatively low OA concentrations (5 mol%) and T_m decreases as a function of OA concentration (Fig. 6). The decrease of T_m suggests that OA causes perturbations in the liposomal lipid bilayer arrangement and fluidity. $\Delta T_{1/2}$ is also dependent on OA concentration (Fig. 7), an increase in OA:DPPC molar ratio leading to a broadening of the phase transition.

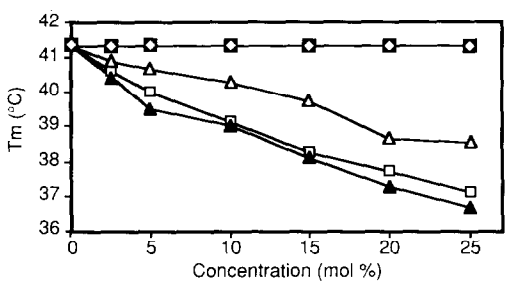

Fig. 6. Transition temperature of DPPC liposomes as a function of amount of compound incorporated into the phospholipid bilayer. (■) Thiamorpholine-3-one; (◇) DMSO; (▲) Azone; (□) C12-thiamorpholine-3-one; (△) oleic acid.

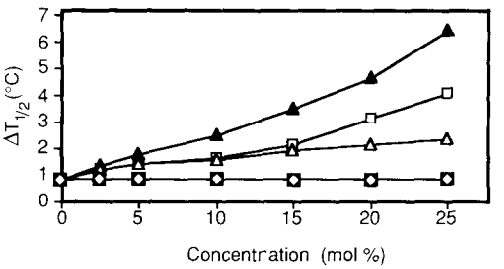

Fig. 7. Influence of the concentration of the tested molecules on the width at half-height of the main DSC peak. (■) Thiamorpholine-3-one; (◇) DMSO; (▲) Azone; (□) C12-thiamorpholine-3-one; (△) oleic acid.

In another study, OA was found to cause fluidisation of the phospholipid hydrocarbon chains of DPPC liposomes and to introduce membrane disorder by intercalating between adjacent DPPC molecules (Gay et al., 1989). Oleic acid has a *cis* double bond in the middle of its hydrocarbon chain so that it has a kinked structure. As a consequence, when intercalated between the DPPC molecules with its polar end close to the phospholipid polar end, OA probably disrupts the ordered packing of the phospholipid molecules, thus increasing liposomal bilayer fluidity.

Dimethyl sulphoxide (DMSO)

As observed in Figs 6 and 7, up to a molar fraction of 25 mol%, DMSO has no significant effect on the phase transition of DPPC liposomes. Even by increasing the DMSO:DPPC molar ratio up to 60 mol%, no modification of the transition temperature (T_m) and of $\Delta T_{1/2}$ was detected (data not shown). It was also demonstrated previously that, in terms of interactions with stratum corneum, DMSO action arises at high concentrations (Barry, 1987; Goodman and Barry, 1989; Khan and Kellaway, 1989).

The relatively low interaction of DMSO with liposomal lipids can be related to the polar nature of this solvent, which probably interacts with the lipid polar head group without partitioning into the lipid bilayer. In that particular case, DSC of DPPC liposomes can represent a precious tool for evaluating and understanding the effect of a solvent, such as DMSO, on lipid bilayers and

consequently it may help for the interpretation of similar effects that could be observed with stratum corneum lipids. However, DPPC liposomes cannot be used for predicting the penetration enhancement efficacy of DMSO, since this accelerant acts better on proteins than on lipids and has in addition a solvent effect on many drugs.

N-Alkylthiamorpholine-3-ones

As part of a continuing effort for understanding structure/activity relationship between penetration enhancers, two chemically related compounds, *N*-alkylthiamorpholine-3-ones, were synthesized (Fig. 1). Based on literature results obtained with *N*-alkylazacycloheptan-2-ones with a varying number of carbon atoms in the alkyl chain (Bouwstra et al., 1989), the enhancing activity of *N*-dodecylthiamorpholine-3-one (C12-thiamorpholine-3-one) was compared to that of thiamorpholine-3-one. As seen in Figs 6 and 7, C12-thiamorpholine-3-one and thiamorpholine-3-one do not modify the gel-to-liquid crystalline transition temperature of DPPC liposomes to the same extent; an increase in the C12-thiamorpholine-3-one proportion shifts the transition temperature to lower temperatures and increases $\Delta T_{1/2}$ whereas thiamorpholine-3-one has no effect on T_m and $\Delta T_{1/2}$.

Since the modifications of T_m and $\Delta T_{1/2}$ induced by C12-thiamorpholine-3-one concentrations are very similar to those obtained with Azone (Figs 6 and 7), C12-thiamorpholine-3-one very likely acts by partitioning directly into the liposomal lipid bilayer structure and by modifying its organisation and fluidity.

The presence of an alkyl chain of specific length in the thiamorpholine-3-one structure appears to be necessary for penetrating the hydrophobic core of the liposomal bilayer and perturbing its fluidity. The lack of alkyl chain prevents the intercalation of the thiamorpholine-3-one into the liposomal bilayer, no perturbation in its organisation being thus obtained.

Conclusions

Most of the presumed penetration enhancers investigated in the present work have been proved, by differential scanning calorimetry, to modify the phase transition temperature of multilamellar dipalmitoylphosphatidylcholine liposomes, used as a lipid bilayer model. Azone, OA and C12-thiamorpholine-3-one induced perturbations of the liposomal lipid bilayer structure, the increase of penetration enhancer:DPPC molar ratio leading to the vanishing of the pretransition peak, to the reduction of the gel-to-liquid crystalline transition temperature and to an increase in the width at half-height of the main DSC peak. Owing to their structure, these compounds very likely intercalate between the phospholipid hydrocarbon chains, perturbing the liposomal bilayer organization and thus increasing its fluidity.

Since at least Azone and OA are known to interact with stratum corneum lipids in a same manner, thereby allowing drugs to permeate more readily through a more fluid lipidic structure, it may be suggested that DPPC multilamellar liposomes could be a suitable model of the structured intercellular lipids of the stratum corneum. Analysis of the phase transition of DPPC liposomes by DSC might therefore represent a useful tool for investigating the mode of action of penetration enhancers and for designing new enhancer molecules.

However, neither DMSO nor thiamorpholine-3-one modified the phase transition temperature of DPPC liposomes. The non-reactivity of DMSO towards the liposomal phospholipid bilayers can be explained by the well-known fact that this penetration enhancer should not significantly partition into lipid chains (Barry, 1987). In this particular case, the test proposed in this study is not powerful enough for predicting whether or not a new molecule will act as a penetration enhancer. Only part of the mode of action of penetration enhancers, i.e., perturbations in the liposomal lipid bilayer structure, can be evaluated using DPPC liposomes and DSC.

When comparing a series of chemically related compounds, the effect of the length of hydrocarbon chains capable of penetrating the hydrophobic core of the lipid bilayer can be evaluated. Indeed, when comparing thiamorpholine-3-one and *N*-dodecylthiamorpholine-3-one, a C12 alkyl chain length was proved to be necessary to achieve

modification of the liposome fluidity by insertion of the penetration enhancer molecule into the lipid bilayer structure. Such a test could be of great interest for selection of new penetration enhancers in a series of molecules differing, for instance, only in their alkyl chain length. Nevertheless, caution should be exercised in extrapolating results from in vitro studies using a liposomal bilayer model to penetration studies.

The effect of these different molecules on the in vitro penetration kinetics of a retinoid-like drug is actually under investigation. Preliminary results appear to indicate a relevant predictivity of the test used in the present work: in the case of *N*-alkylthiamorpholine-3-ones, for instance, a structure-activity effect comparable to that obtained by DSC is observed in the penetration studies.

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