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Interaction of phospholipid liposomes with lipid model mixtures for stratum corneum lipids

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Summary

Differential scanning calorimetry (DSC) and ^{2}H-NMR experiments were performed on lipid vesicles prepared from a lipid mixture resembling the lipid composition of stratum corneum lipids in the epidermis. This lipid mixture showed a broad phase transition with a transition range from 40 to approx. 80°C. ^{2}H-NMR spectroscopy revealed that the transition occurs from a lamellar gel-like phase to a cubic phase at high temperature. Both phases coexist over a broad temperature range. Mixing of populations of different soy bean phosphatidylcholine or dipalmitoylphosphatidylcholine vesicles with the vesicles made from the model mixture for stratum corneum lipids resulted in the mixing of lipid components, induced either by heating the vesicles to 95°C, or by incubation of the vesicle mixtures at 37°C for 2–24 h. The mixing of lipid components apparently proceeds by monomer exchange through the water phase. ^{2}H-NMR spectroscopy showed that the resulting mixed lipid system has different phase characteristics, being in a liquid-crystalline state at 37°C and transforming apparently to an inverted hexagonal phase at higher temperature. The consequences for the penetration of liposomes through the stratum corneum of the skin are discussed. Mixing of liposomes with the lipids in the intercellular layers could be one mechanism contributing to the enhancement of the permeability of the skin to lipid vesicles.

Introduction

There is increasing evidence that liposomes have a great potential as drug delivery systems, not only for intravenous delivery, but particularly for topical applications. In addition, beneficial effects on the skin itself could be demonstrated (Ghyczy and Niemann, 1992). Liposomes are one of the most widely used model systems for biological membranes and the ways in which liposomal membranes interact with cell membranes and other model membrane systems have been studied in great detail. In particular, the fusion of vesicle membranes induced by a variety of fusogenic agents, such as lysolipids, long chain alcohols, fatty acids, viruses, and multivalent cations has been studied, also focussing on the question of whether fusion takes place without leakage of vesicle contents (for a review, see Prestegard and

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O'Brien, 1987). Recently, several publications have appeared which deal with the uptake of phospholipids from liposomes into the stratum corneum (Lasch et al., 1991; Röding and Artmann, 1992), and the successful transport of lipophilic and hydrophilic compounds, proteins, and other macromolecules from topically applied liposomes into the skin (Cevc, 1992a,b; Cevc and Blume, 1992; Planas et al., 1992) (for reviews, see Egbaria and Weiner, 1990; Schubert, 1991; Ghyczy and Niemann, 1992). The molecular basis for penetration enhancement, the mechanism of interaction of liposomes with the epidermal lipid barrier, and penetration properties of liposomes are still not completely clear and are being intensively discussed (Foldvari et al., 1990; Lasch et al., 1991; Cevc, 1992a; Cevc and Blume, 1992; Planas et al., 1992).

The permeability barrier of the skin, which prevents penetration of substances from the environment is localized in the horny layer (stratum corneum), which is a compact mass of metabolically inactive cells, embedded in an extracellular matrix of non-polar continuous lamellar lipid layers. These lamellar lipid layers are viewed as the major obstacle against the penetration of exogenous substances through the skin (for reviews, see Elias, 1991; Landmann, 1991).

Recently, it was suggested that one possible prerequisite of the penetration enhancement properties of phospholipid liposomes could be the fusion of the vesicle bilayers with the lipid layers of the stratum corneum. As a result of this fusion, the lipid barrier of the skin would be altered in three ways. Because of the changed chemical composition, the lipid barrier could become more fluid and, secondly, more hydrophilic. Thirdly, the continuous lipid barrier could be disrupted by forming flattened vesicles, structures which are found in the lipid granules, situated in the stratum granulosum (Ghyczy, 1992). Also, the lipid mixture resulting from the fusion process could be 'H_{II}-prone', forming 'interlamellar attachments' (ILA) or 'inverted micellar intermediates' (IMI), stages which are thought to be intermediates between lamellar and hexagonal or cubic phases (Siegel et al., 1989; Tate et al., 1991; Tournois and De Kruijff, 1991). In both cases the formation of gaps would enable the entry of substances applied on the skin into the skin.

The aim of our work was to show that interactions of phospholipid liposomes with liposomes composed of a model mixture for stratum corneum lipids do occur and that the interaction leads to systems in which the phospholipids are mixed with the model lipids for stratum corneum lipids. This process was followed by differential scanning calorimetry and, in more detail, by ^{2}H-NMR spectroscopy. The suggested mechanism for the formation of gaps in the lipid barrier of the skin in vivo is thus supported by our experiments. The resulting disruption of the continuity of the lipid barriers might then permit the penetration of intact liposomes through the stratum corneum (Cevc, 1992a,b; Cevc and Blume, 1992).

Materials and Methods

Dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), cholesterol, cholesterol sulfate, palmitic acid, and ceramide type III prepared from sphingomyelin from bovine brain were purchased from Sigma (Deisenhofen, Germany) and used without further purification. Perdeuterated palmitic acid (98%) was a product of Cambridge Isotope Laboratories, Woburn, MA, U.S.A. Phospholipon 80 and Phospholipon 90/90G were products of Nattermann Phospholipid GmbH, Cologne, Germany. These are soy bean lipid extracts with approx. 80 and 93% content of phosphatidylcholine, respectively.

Lipid vesicles for DSC experiments were prepared by sonication of the lipid in 20 mM phosphate buffer (pH 7.0) in bath type sonicator (Branson 1200) at temperatures between 60 and 80°C for approx. 20 min in a small closed glass vial holding 10 ml vesicle suspension. The resulting vesicle preparation was annealed for another 30–60 min at 80°C and then immediately cooled. The diameter and the size distribution of the vesicles were determined by dynamic laser light scattering (DLS) using a Malvern Zetasizer 3. The autocorrelation function was analyzed using the Malvern software, applying the cumulant and multi-exponential fit methods. The average size

of the vesicles in the case of DPPC, Phospholipon 80 and 90/90G, and the model mixture for stratum corneum lipids was between 100 and 150 nm.

The stratum corneum model lipid mixture consisted of 40 wt% ceramide, 25 wt% cholesterol, 25 wt% palmitic acid, and 10 wt% cholesterolsulfate (Wertz et al., 1986). Mixing was accomplished by first dissolving the lipids in $CHCl_3$/MeOH and after complete dissolution removing the solvent in a stream of nitrogen. Residual solvent was then removed in oil pump vacuum for at least 12 h. To the resulting lipid film 20 mM phosphate buffer (pH 7.0) was added and the sample sonicated as described above. The vesicle suspension for stratum corneum lipids was quite stable, the average size after 18 days of storage increasing only by approx. 10%.

Differential scanning calorimetry (DSC) was performed using a Microcal MC 2 DSC instrument. The heating rate was 1°C/min, and lipid concentrations were between 1 and 2 mg/ml.

^{2}H-NMR experiments were performed using a Bruker AMX-400 NMR spectrometer at a ^{2}H frequency of 61.4 MHz. The ^{2}H probe was a Doty Scientific ^{2}H solid state NMR probe with a 5 mm solenoid. The low power output of the Bruker transmitter was used to drive a 1 kW high power amplifier (Doty Scientific, Inc.). The quadrupole echo sequence with phase cycling, a pulse separation of 40 μs and a $\pi/2$ pulse of 2.4 μs was used. 5000–10000 echos with a dwell time of 3.3 μs were accumulated using a recycling delay of 200 ms. Spectra were aquired at different temperatures using a Bruker variable temperature control unit. Before each acquisition was started the temperature was allowed to equilibrate for at least 20 min. The skin lipid model mixture for the NMR experiment consisted of a total of 24 mg of lipids taken up in 50 μl of deuterium depleted water (Aldrich, Steinheim, Germany).

Results and Discussion

DSC experiments

Our inital experimental approach to study the interaction of phospholipid vesicles with a model mixture for stratum corneum lipids was to use differential scanning calorimetry to follow the characteristics of the thermally induced phase transition before and after mixing the model skin lipids with equimolar amounts of DPPC, Phospholipon 80, or Phospholipon 90/90G vesicles, respectively. As the soy bean lipids contain mainly phosphatidylcholine with highly unsaturated chains (approx. 60–70% linoleic acid), the thermal phase transition from the gel to liquid-crystalline phase for these lipids has a transition temperature far below 0°C and could not be detected with our adiabatic DSC instrument. The question was then whether our model mixture for stratum corneum lipids would show a phase transition detectable by DSC and whether changes in the phase transition behaviour could be induced by the addition of phospholipid vesicles. Mixing of lipids of different vesicle populations can occur either by fusion of vesicles to larger structures or by lipid exchange via monomer diffusion through the water phase. In both cases a change in the thermotropic behaviour should be detectable by a difference in the DSC thermograms.

The DSC curve of our model mixture for skin lipids is shown in Fig. 1a. A broad endothermic event between 40 and 90°C is observed, with an apparent maximum at approx. 70°C. The DSC curve was essentially unchanged and reproducible in the second and third heating scans. Broad transitions are characteristic for lipid mixtures containing cholesterol (Demel and De Kruijff, 1976; Blume, 1980; Yeagle, 1985). Overall, our model mixture contains approx. 36 mol% cholesterol, including the cholesterol sulfate. From the DSC curve it is not clear between which states the transition is occurring, although one can assume that at low temperature the mixture is in a lamellar state. Similar results for lipid mixtures of this type have been obtained previously and it has also been reported that these mixtures are able to form stable liposomes in agreement with our findings (Wertz et al., 1986; Potts et al., 1991; Abraham and Downing, 1992). This is only possible when the lipids are in a bilayer state. The transition enthalpy could not be determined due to the relatively low signal-to-noise ratio for this dilute vesicle suspension, which makes the correct de-

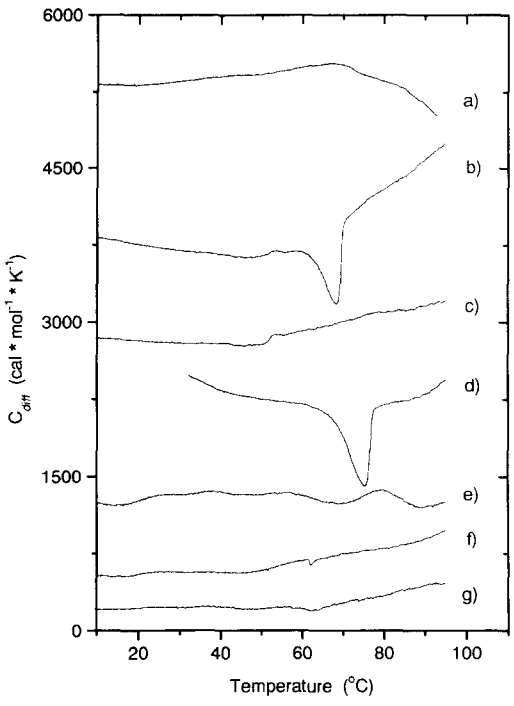

Fig. 1. (a) Calorimetric scan of stratum corneum model lipids; (b) first and (c) second calorimetric scans after mixing with equimolar amount of a Phospholipon 80 vesicle suspension after incubating the sample at 2°C after mixing; (d) first and (e) second heating scans measured after incubation of the sample for 6.5 h at 20°C after mixing; (f) first and (g) second heating scans after incubation of the sample at 37°C for 4 h.

termination of the beginning and end of the transition impossible.

After the first heating scan the model stratum corneum lipids were mixed at room temperature with an equimolar amount of Phospholipon 80 vesicles. The calorimeter cell was filled with the vesicle mixture, cooled to 2°C and after 60 min equilibration immediately scanned to a temperature of 95°C. The resulting DSC curve is shown in Fig. 1b. At approx. 70°C an exothermic event is observed, indicating that the dispersion was in a metastable state after mixing of the two vesicle populations and could reach a more stable state by an as yet unidentified exothermic process. The third scan (Fig. 1c) was obtained after cooling the sample to 2°C and immediately heating again. No further exotherm is observed.

We repeated these experiments, but now equilibrated the sample at 20°C for 6.5 h after mixing the two vesicle populations. The first and second scan after this equilibration period are shown in Fig. 1d and e. Again the first heating scan shows an exotherm, this time at approx. 75°C, which has disappeared in the second heating scan.

In a third experiment we incubated the sample at 37°C for 4 h after mixing the two suspensions of the Phospholipon 80 and the stratum corneum model lipid vesicles. The first and second heating scans are shown in Fig. 1f and g, respectively. In this case no exothermic event was observed in the first scan after the equilibration period, essentially only baselines being recorded.

The experiments were repeated with the other soy bean phospholipid extract, Phospholipon 90/90G, which is more enriched in phosphatidylcholine. Essentially similar results were obtained, only that the exotherm observed after equilibration at low temperatures (20°C or below) was now centered at somewhat higher temperature (80–85°C). Incubation at 37°C for 5–10 h after mixing was sufficient to eliminate the exotherm observed in the first heating scan after mixing and equilibration at low temperature.

Since both phospholipid mixtures show no phase transition above 0°C and the stratum corneum model lipid mixture displayed only a broad, ill-defined transition, interpretation of the results of the DSC experiments is difficult. Dipalmitoylphosphatidylcholine (DPPC) undergoes a phase transition at 41.3°C from the gel to the liquid-crystalline phase in multi-lamellar systems (for a review, see Blume, 1991). For vesicles of 100–150 nm diameter this transition is only slightly lowered and somewhat broadened (not shown). We repeated our mixing experiments with DPPC vesicles, as in this case changes of the DPPC transition endotherm should be observed if mixing of lipids within bilayers occurs. Fig. 2 shows the results of the DSC experiments.

The first heating scan (Fig. 2a) immediately after mixing the DPPC vesicles with the stratum corneum vesicles shows that considerable mixing of lipid components has already taken place after the equilibration period. The maximum of the endothermic peak has shifted to 52°C. Several

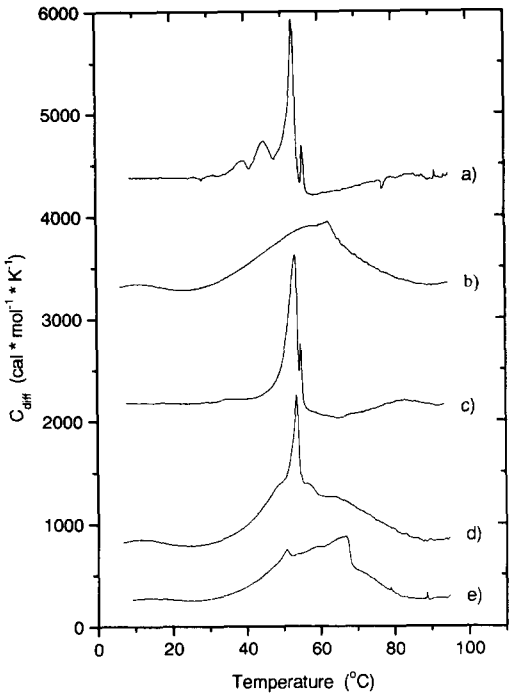

Fig. 2. Calorimetric scans of stratum corneum model lipids after mixing with an equimolar amount of DPPC vesicles: (a) first and (b) second heating scans measured immediately after mixing the vesicle populations; (c) first heating scan after incubation of the sample for 3 h at 37°C after mixing; (d) first heating scan after incubation for 8 h at 37°C after mixing; (e) first heating scan after incubation for 24 h at 37°C after mixing.

small exothermic peaks are superimposed on this endotherm, particularly after the main endothermic peak, indicating the temperature induced formation of a more stable state. The second scan after cooling (Fig. 2b) shows only a broad endotherm with a maximum centered at approx. 60°C. The form of this endothermic peak does not change in subsequent heating scans (not shown). In separate experiments the vesicle mixture was then equilibrated at 37°C for 3, 8, and 24 h. The first heating scans after this equilibration period are shown in Fig. 2c–e. All second heating scans were identical to scan b. The incubation experiments show that the mixing of vesicle components can be achieved either by heating the mixed vesicle populations up to 95°C, or by incubation for approx. 24 h at 37°C. Thus, the results obtained with DPPC vesicles are qualitatively similar to those obtained previously with Phospholipon 80, or Phospholipon 90/90G vesicles, i.e., mixing of vesicle components occurs after long equilibration or after heating. However, the times needed for complete mixing are longer for DPPC vesicles. This is not surprising, since the DPPC vesicles are in the gel state at 37°C, whereas the soy bean phospholipids are in the liquid-crystalline state way above their respective phase transition. Dynamics in the liquid-crystalline phase is much higher.

To evaluate the possibility that the relatively rapid lipid mixing after mixing two separate vesicle populations is a phenomenon characteristic for all phospholipid vesicles, we studied a model system consisting of a DSPC/20 mol% cholesterol mixture. This particular composition was chosen, since the mixture contains cholesterol, has a transition temperature around 50°C and is thus in a gel-like state at 37°C, like our stratum corneum model lipid mixture. Fig. 3a shows the DSC curve for the DSPC/cholesterol mixture. The first and second heating scans after mixing this vesicle population with DPPC vesicles are shown in Fig. 3b and c, respectively. The two peaks corresponding to the transitions of DPPC and DSPC/cholesterol are clearly separated. The two peaks gradually change shape and converge, the maximum of the high temperature peak being shifted to lower temperature. Obviously, no direct fusion of vesicles takes place, as no additional peak at intermediate temperature is observed. Mixing of lipids apparently occurs by asymmetric transfer of lipid monomers through the water phase, DPPC molecules being incorporated more and more into the DSPC/cholesterol vesicles lowering their transition temperature. These findings are in agreement with previous data on lipid transfer (Bayerl et al., 1988). The first and fourth heating scans after incubation for 24 h at 37°C are shown in Fig. 3d and e. This incubation period is apparently insufficient to induce a measurable increase in lipid transfer, as scan d with incubation and scan b without are almost identical. In addition, repeated heating does not lead to complete mixing of lipids. The experiments with this reference system show that

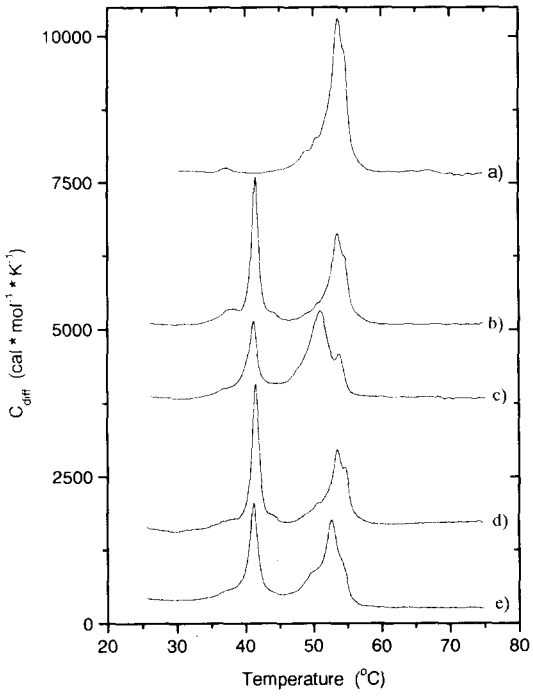

Fig. 3. (a) Calorimetric heating scan of DSPC/20 mol% cholesterol vesicles; (b) first and (c) second scans measured directly after mixing with DPPC vesicles; (d) first and (e) fourth heating scans after incubation of the sample at 37°C for 24 h after mixing.

lipid mixing by monomer transfer is very slow in these model systems. This was quite different in the mixing experiments with stratum corneum model lipids. Here complete mixing could be achieved by 4 h incubation at 37°C in the case of Phospholipon 80, and 24 h incubation for DPPC vesicles. The higher mixing rate is thus obviously due to the particular lipid composition of the stratum corneum model lipid mixture with its high content of fatty acid and charged cholesterol sulfate. In these cases, the DSC experiments cannot distinguish whether mixing occurs via monomer exchange through the water phase or by direct fusion of lipid vesicles. Dynamic light scattering experiments of the mixed vesicle suspensions of DPPC with stratum corneum model lipids revealed only a slight increase in mean vesicle diameter by 10–15% after the fourth heating scan. Thus, the mixing of lipid components is more likely, otherwise a considerable increase in mean diameter should have been observed.

^{2}H-NMR experiments

As DSC provides only indirect evidence for lipid mixing, we performed additional experiments using ^{2}H-NMR spectroscopy. This method allows direct observation of the order, conformation and dynamics of lipid molecules labeled with the deuterium isotope. Transitions between different phases, such as the gel, liquid-crystalline L_α, H_{II}, and cubic phases, can readily be distiguished by changes in line shape (Seelig, 1977; Griffin, 1981; Davis, 1983; Blume, 1988).

To elucidate the nature of the broad endothermic transition observed by DSC for the stratum corneum model lipid mixture (Fig. 1a), we investigated the same mixture as a function of temperature by ^{2}H-NMR using perdeuterated palmitic acid as a nuclear spin probe. Due to the limited sample size the signal-to-noise ratio was relatively low. Fig. 4 shows the ^{2}H-NMR spectra of our

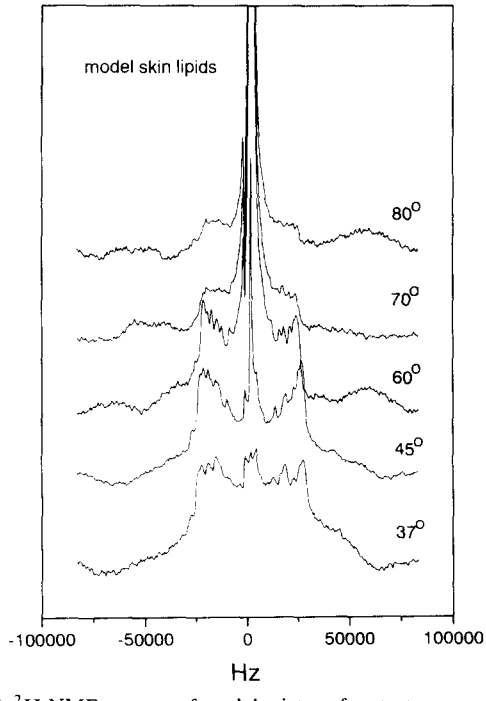

Fig. 4. ^{2}H-NMR spectra of model mixture for stratum corneum lipids prepared with perdeuterated palmitic acid as a function of temperature.

model mixture as a function of temperature. The palmitic acid spectrum at 37°C displays a powder pattern with a maximum splitting $\Delta\nu_{Q_\perp}$ of approx. 54 kHz. In addition, resonances arising from different -CD_2- groups along the chain and the terminal -CD_3 group are partly resolved. The general line shape and width indicate that the palmitic acid molecule in the lipid mixture rotates around its long axis with correlation times shorter than 10^{-6} s, and that some *trans-gauche* isomerization with a probability of 5–10% *gauche* for positions in the middle of the chain is possible (Blume et al., 1982; Blume, 1988). The probability for *gauche* conformers increases towards the chain ends. With increasing temperature the splittings for the individual -CD_2- groups become better resolved, indicating increased rotation rates, and the splittings decrease due to an increase in *gauche* conformers. In the middle part of the spectrum a single line with increasing intensity is observed, being first visible in the spectrum taken at 45°C. This sharp line could be caused by the formation of small vesicles, which are able to perform rapid isotropic tumbling to average the quadrupolar interaction. This can be ruled out in our case, as the concentrated and coarse lipid dispersion used for the NMR experiments showed no sign of a change in morphology, characteristic for the formation of small vesicles. The other possibility is that the palmitic acid molecules are able to perform averaging motions by lateral diffusion in all three directions in their particular phase state. This is possible in cubic phases where the bilayers form an infinitely periodic minimal surface (Gruner, 1989; Larsson, 1989).

The spectra between 45 and 80°C are two-component spectra, the component giving the sharp line, presumably being the cubic component, continuously increases in intensity, while the other component giving the powder pattern characteristic for the lamellar phase decreases in intensity. At 60°C the maximal splitting is still 48 kHz, indicating that the lamellar phase is still gel-like, or resembles the so-called 'liquid-ordered' phase found in phospholipid/cholesterol mixtures (Ipsen et al., 1987). In DPPC/25 mol% cholesterol bilayers at 38°C the maximal splitting for perdeuterated DPPC is approx. 53 kHz and thus comparable to the values we determined for palmitic acid in the mixture (Vist and Davis, 1990).

The phase transformation we observe by ^{2}H-NMR is thus apparently from a gel-like liquid-ordered lamellar state to a cubic phase. Abraham and Downing (1992) have investigated similar model mixtures for stratum corneum lipids by ^{2}H-NMR and found a transition from a lamellar to an inverted H_{II} phase above 60°C. We did not observe the distinct appearance of splittings characteristic for the hexagonal phase, but we cannot rule out the possibility that they occur as intermediates in the transition to the cubic phase. The signal-to-noise ratio of the spectra in the two-component region is relatively low. This is characteristic for two-phase regions, when exchange processes with correlation times of the size of the quadrupole echo sequence are present. This leads to a strong reduction of T_{2E} with a concomitant decrease in echo intensity (Blume et al., 1982). However, the differences between our results and those of Abraham and Downing could also be caused by the different lipid compositions used, as our mixture contained more palmitic acid. The results of our DSC and ^{2}H-NMR experiments can therefore be explained by postulating a phase transition from a gel-like lamellar phase to a cubic phase, the braod transition region extending from 45 to more than 80°C.

The mixing experiment of stratum corneum model lipids with Phospholipon 80 vesicles was now repeated using ^{2}H-NMR as the detection method. The model lipid mixture containing the perdeuterated fatty acid was mixed in the NMR sample tube with the same molar amount of Phospholipon 80 dispersion in approx. 50–70 μl deuterium depleted water, giving a total concentration of 63 mg of lipid in approx. 150 μl water. The concentrated lipid suspension was stirred with a syringe needle in the NMR sample tube and then immediately introduced into the solenoid of the ^{2}H probe. Fig. 5 shows the ^{2}H-NMR spectra after various times of incubation at 37°C in comparison to the spectrum of the model mixture before mixing with Phospholipon 80 (lowest trace). After 1.5 h of equilibration the

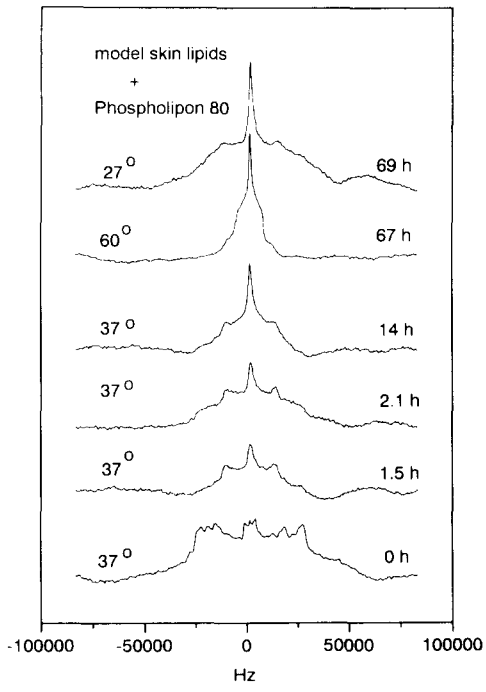

Fig. 5. ^{2}H-NMR spectra of model mixture for stratum corneum lipids prepared with perdeuterated palmitic acid before and after mixing with Phospholipon 80 vesicles. The different times after mixing and the temperatures are indicated. The lower spectrum is that of the mixture before addition of Phospholipon 80.

line shape has markedly changed. The spectrum after 14 h incubation is only slightly different from the first spectrum taken after 1.5 h, indicating that at this higher lipid concentration used in the NMR experiments the mixing of the stratum corneum model lipids with the Phospholipon 80 is essentially complete after approx. 2 h. The line shape indicates a transformation into a phase with increased disorder. The width of the powder pattern is now only 27 kHz, showing that the amount of *gauche* conformers has increased. However, the splittings of individual -CD_2- groups along the palmitic acid chain are not resolved. An increase in temperature to 60°C leads to a considerable reduction in spectral width, roughly by a factor of two. Possibly a transition into the H_{II} phase now takes place. Cooling the sample to 27°C leads to the appearance of a line shape without resolved splittings similar to the first and second spectra recorded at 37°C after mixing. The width of the spectrum remains smaller than that of the pure stratum corneum model lipids.

The ^{2}H-NMR spectra thus provide evidence for a change of the composition of the lipid matrix as detected by the palmitic acid probe molecule. The morphology and structure of the lipid dispersion may be complex. Several structures, such as lamellar phase, H_{II} phase and other intermediates may coexist, the palmitic acid molecule sampling each of these different structures. Exchange of the fatty acid molecule between these different surroundings giving different line shapes could lead to the observed loss of resolution of the splittings of the individual -CD_2- groups, particularly when the exchange rates are close to the intermediate time regime of 10^{-5}–10^{-6} s. The results of the NMR experiments are thus in agreement with our DSC results, namely, that lipid mixing occurs after different vesicle populations have been added, and that at 37°C this mixing of phospholipids with stratum corneum model lipids is relatively fast, with half-times between 3 and 8 h, depending on the type of phospholipid used and on the lipid concentration.

Summary and Conclusions

We have shown that DSC and ^{2}H-NMR can be used to study the interactions of lipids of different vesicle populations. Our results with stratum corneum model lipids reveal that intermixing with phosphatidylcholines with saturated and unsaturated chains occurs, induced either by heating the mixed sample to 95°C, or by incubation at 37°C for 2–24 h, the longer times being needed for the saturated DPPC. This process is rapid compared to lipid exchange processes observed with DPPC and DSPC/cholesterol vesicles where, even after the extended periods of incubation and several heating cycles, complete intermixing was not observed. The mechanism by which lipid intermixing takes place seems to be monomer exchange via the water phase, at least in the case of DPPC mixed with DSPC/cholesterol. Other mechanisms, such as direct fusion of

vesicles of different lipid composition, cannot be ruled out for the mixtures with stratum corneum model lipids, although preliminary experiments with dynamic light scattering showed no marked increase in vesicle size. Due to the specific composition of this model mixture with its high content of fatty acid and cholesterol sulfate, the mixing by monomer exchange seems more likely, as the rate of this process is determined by the monomer solubility in water, which is much higher for palmitic acid and cholesterol sulfate than for lipids such as DPPC or DSPC.

The question as to whether intact liposomes can penetrate into the skin is still a matter of debate. Recently, it was shown that lipid vesicles can indeed reach deeper layers of the skin and can even reach the blood compartment. (Cevc, 1992a; Cevc and Blume, 1992; Planas et al., 1992). The driving force for this phenomenon was suggested to arise from transdermal osmotic gradients, as the outer layers of the epidermis have a much lower water content. A prerequisite for the penetration of lipid vesicles into the skin is then that the lipid vesicles are flexible enough to squeeze through 'holes' in the intercellular lipid layers, and that the driving force is not abolished by occlusion of the skin surface, where the lipid vesicles are applied (Cevc and Blume, 1992). Our experiments show that the interaction of phospholipids with stratum corneum model lipids leads to a mixing of lipid components. This could also occur in vivo and lead to the formation of more hydrated lipid layers, which then could lead to a structural rearrangement of the stratum corneum lipids in such a way as to form stacked, more flattened vesicles as found in the stratum granulosum. Another possibility is the local formation of lipid compositions in the layers, which are 'H_{II}-prone', i.e., on the verge of forming inverted hexagonal phases (Siegel et al., 1989; Tate et al., 1991; Tournois and De Kruijff, 1991). As a result, interlamellar attachments could form leading to holes in the lipid layers. Once these structures are formed, other lipid vesicles could then squeeze through these defects reaching the lower layers of the skin. While this mechanism for the penetration of lipid vesicles into the skin is still highly speculative, it is clear that according to our results the possibility for local mixing of phospholipids from the applied vesicles with the stratum corneum lipids should be taken into account. This mixing process, either via monomer exchange or by direct fusion, is strongly dependent on the phase state, the composition of the applied liposomes, and the composition of the resulting mixed lipid layers. Thus, it is understandable that different liposome formulations can have different effectiveness in penetrating the skin. A thorough physico-chemical analysis of the possible events occurring after mixing of different lipid dispersions is thus a prerequisite for the understanding of the processes taking place in vivo. The experiments we performed on model systems are therefore one means of obtaining more information on these processes. A systematic study using liposomes with different composition should show ways in which to optimize the lipid composition of a liposomal formulation designed to transport drugs into or through the skin.

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