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Ethanol effects on the stratum corneum lipid phase behavior

Steven L. Krill [a], Kristine Knutson [b] and William I. Higuchi [b]

[a] *Abbott Laboratories, Pharmaceutical Product Division, North Chicago, IL (USA) and* [b] *Center for Controlled Chemical Delivery, Department of Pharmaceutics, University of Utah, Salt Lake City, UT (USA)*

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The stratum corneum is considered to be the diffusional barrier of mammalian skin for water and most solutes. The intercellular lipid multilayer domains of the stratum corneum are believed to be the diffusional pathway for most lipophilic solutes. Fluidization of the lipid multilayers in the presence of ethanol is frequently conceived to result in enhanced permeation. Current investigations address the effect of ethanol on the phase behavior in terms of stratum corneum lipid alkyl chain packing, mobility and conformational order as measured by Fourier transform infrared (FTIR) spectroscopy. Phospholipid multilamellar vesicles were also studied as model systems. There appeared to be no effect of ethanol on either the solid–solid phase transition or the gel phase interchain coupling of the stratum corneum lipids. However, there was a reduction in the mobility of the alkyl chains in the presence of ethanol. Possible mechanistic relationships between the current FTIR spectroscopic results with available literature data of ethanol induced lipophilic solute penetration enhancement through the skin are discussed.

Introduction

Current interest in transdermal delivery of medicinal agents into systemic circulation has prompted the use of solvents (penetration enhancers) to reduce the barrier properties of the skin. The outer layer of the skin, the stratum corneum, exhibits the greatest resistance to diffusion through skin [1–5]. The diffusional pathway through the stratum corneum is dependent on the physicochemical properties of the diffusing solute, whereby nonpolar solutes permeate through a lipophilic domain and polar solutes diffuse through a more polar environment [1–5]. The ordered multilayer structure of the stratum corneum interstitial lipids is proposed to control the permeation of nonpolar solutes through the skin [3]. The thermally dependent increased permeation of n-alkanols [1,4,5] and hydrocortisone [4,5] through the stratum corneum is believed to result from fluidization of the lipid multilayers. Knutson et al. [5] have also demonstrated an abrupt permeation enhancement of selected solutes through the hairless mouse stratum corneum lipids during the gel to liquid-crystalline phase transition.

Correspondence to: K. Knutson, Department of Pharmaceutics/CCCD, University of Utah, 421 Wakara Way, Salt Lake City, UT 84108, USA.

Several solvents or penetration enhancers, such as oleic acid [6], dimethylsulfoxide [7] and Azone^{R} [8] have demonstrated membrane perturbing effects resulting in permeation enhancement through the skin at physiologic temperature. Recently, Higuchi et al. [9] have shown the enhancement of β-estradiol and hydrocortisone through hairless mouse skin in the presence of ethanol. It was concluded in the latter study that ethanol enhances permeation by fluidizing the interstitial lipid multilayers, thus reducing the diffusional resistance of the pathway. Tillotson et al. [10] demonstrated the enhanced efflux of the fluorescent dye, 6-carboxyfluorescein, from rat intestinal microvillus membrane vesicles in the presence of ethanol. However, an ethanol induced increase in membrane lipid fluidity was not detected.

Lipid fluidization effects by ethanol have been observed in model lipid membranes and biomembranes with spin probe [11–16] and fluorescence depolarization [13,17–20] measurements. The thermotropic phases of the lipid membranes are not identified in many of the aforementioned investigations [11–13,15,17,19,20]. Most biomembranes are in the liquid-crystalline phase above room temperature. Therefore, the results reported are assumed to reflect the ethanol effects on membranes in the liquid-crystalline phase. These results agree with the ethanol fluidizing effects of lipid membranes in the liquid-crystalline phase re-

ported by Pang et al. [14], Chin and Goldstein [16] and Harris et al. [18].

Studies of ethanol effects on lipid membranes in the gel phase are more limited. A reduction in the gel to liquid-crystalline phase transition temperature of phospholipid bilayers in the presence of ethanol has been reported [21–24]. Rowe [21,22] has demonstrated an ethanol concentration dependent decrease followed by an increase in the transition temperature of model phospholipid bilayer membranes. It was concluded that ethanol at low concentrations preferentially acts on the fluid region of the bilayer resulting in a freezing point depression. At higher concentrations, ethanol was believed to stabilize the gel phase. The X-ray diffraction studies of Simon and McIntosh [25] have shown that high concentrations of ethanol induce an interdigitated gel phase in DPPC bilayer membranes under the same experimental conditions as the investigations reported by Rowe [21,22].

Thus, the effects of ethanol on the lipid membranes are dependent on the phase of the membrane. Skin permeation studies support the concept of ethanol induced lipid fluidization [9]. A relationship between thermal fluidization of the stratum corneum lipid alkyl chains with alterations in the gel phase packing, mobility and conformational order and thermally enhanced permeability has been previously established [26]. The current investigation addresses the specific influence of low ethanol concentrations on the stratum corneum lipid multilayer thermotropic phase behavior and the lipid structural alterations below the solid–solid phase transition (~ 43°C). The altered transport of solutes through the stratum corneum is discussed in relation to the observed ethanol mediated effects on the stratum corneum lipid multilayer structural properties.

Materials and Methods

Stratum corneum preparation

Male hairless mice (SKH-HR-1 strain, Skin Cancer Hospital, Temple University, Philadelphia, PA) between 56 and 200 days old were used as a source of abdominal stratum corneum. A detailed description of the enzymatic separation of the stratum corneum from the epidermis has been previously reported [4]. After vacuum desiccation, the stratum corneum abdominal sheets were stored in desiccators over Drierite^{R} (W.A. Hammond Drierite, Xenia, OH) until used.

Stratum corneum samples were rehydrated by suspending them in a chamber with vapor in equilibrium with deuterium oxide (D_2O, Sigma, St Louis, MO) or deionized water (H_2O) for 20 to 42 h. All D_2O-treated sample manipulations were performed in a nitrogen atmosphere to prevent deuterium exchange with atmospheric hydrogen.

Distearoylphosphatidylcholine / distearoylphosphatidic acid (DSPC / DSPA) and dipalmitoylphosphatidylcholine / dipalmitoylphosphatidic acid (DPPC / DPPA) multilamellar vesicle (MLV) preparation

The DSPC, DSPA, DPPC and DPPA phospholipids (Sigma, St. Louis, MO) were used as obtained from the supplier. The vesicle preparation technique has been described in detail elsewhere [27]. Briefly, the lipids were dissolved in chloroform to form a 96:4 (DSPC/DSPA or DPPC/DPPA) molar ratio. The solution was kept at −4°C until used. A quantity of lipid chloroform solution was pipetted into a 500 ml round bottom flask and placed on a rotating evaporator (Buchi, Switzerland) with the water bath maintained at 70°C and 50°C for the DSPC/DSPA and DPPC/DPPA suspensions, respectively. These temperatures are well above the phospholipids' gel to liquid-crystalline phase transitions. A stream of nitrogen was passed over the solution during the evaporation of chloroform resulting in the deposition of a thin lipid film onto the walls of the flask. The lipid film was vacuum dried (10^{-4} Torr) for one to two hours to remove the residual chloroform. Under a nitrogen atmosphere, 5 ml of D_2O were added to the flask giving a final lipid concentration of 50 mM. The suspension was rapidly rotated (approx. 75 rpm) at the selected temperature until a 'milky white' suspension was formed (approx. 15 min). The MLV suspension was placed in a vial under nitrogen and cooled slowly overnight to room temperature. A 30% v/v perdeuterated ethanol/MLV (CD_3CD_2OD, Sigma, St. Louis, MO) suspension was prepared in a similar manner and allowed to equilibrate overnight.

The preparation of electron microscopic samples from vesicle suspensions is described by Forte and Nordhausen [28]. Samples of the DSPC/DSPA vesicle suspension with and without ethanol were examined at a magnification of 14550X with a JEM 100CXII transmission electron microscope (JEOL, Peabody, MA). The presence of multilamellar vesicles under both experimental conditions was established. No other structures were observed.

Stratum corneum thermal perturbation experiments

The rehydrated samples were immersed in a solvent (D_2O, 20% (v/v) perdeuterated ethanol/D_2O, 36.7% (v/v) perdeuterated ethanol/D_2O or 75% (v/v) ethanol (CH_3CH_2OH)/H_2O) (200 proof, ethyl alcohol, USI, Tuscola, IL) for 6 h. The samples were sealed between two zinc selenide discs and placed in a Barnes-SpectraTech (Stamford, CT) temperature controlled cell. Isothermal conditions were maintained (±0.25°C) with an Omega (Stamford, CT) temperature controller.

Absorbance spectra (0.5 cm^{-1} resolution, 256 scans, noise level of 0.1, zero fill factor of two and triangular apodization) were obtained with a Digilab FTS 20/80

(Cambridge, MA) Fourier transform infrared (FTIR) spectrometer equipped with a nitrogen cooled, narrow band MCT detector over the 3900 to 900 cm^{-1} region. Individual spectra were recorded at selected temperatures between 30 and 80°C under isothermal conditions over a 4-h period.

Membrane reuse experiments

The rehydrated stratum corneum was placed on a 25 mm zinc selenide disc wetted with D_2O and inserted into a H rick (Harrick Scientific, Ossining, NY) sample holder. Two 50 μm polyethylene half-spacers were placed around the sample. Additional D_2O was added prior to sealing the sample between the two discs. The sample was allowed to equilibrate with D_2O for 2–3 h. A spectrum (2.0 cm^{-1} resolution, 1024 scans, zero fill factor of four, noise level 0.1 and triangular apodization) was obtained at 29°C. The D_2O was replaced in the void space between the two zinc selenide discs by initially flushing with 0.5 ml of a 30% v/v perdeuterated ethanol/D_2O solution through the ports of the sample holder. This was followed by 0.2 ml flushes every 10 min for a total of 2 h. A second spectrum was obtained of the sample under identical spectral conditions.

Vesicle thermal perturbation studies

Thermal perturbation FTIR experiments were performed in a similar manner as previously described. A drop of MLV suspension was sealed between two zinc selenide crystals incorporating a 50 μm polyethylene spacer. The sample was placed in the controlled temperature cell and spectra (1 cm^{-1} resolution, 256 scans, zero fill factor of two, noise level 0.1 and triangular apodization) were recorded under isothermal conditions at selected temperatures between 30 and 70°C during a 3-h period.

The data presented are averages of three samples (unless otherwise stated) ± the standard deviation.

The wavenumber positions of the CH_2 asymmetric and symmetric stretching vibrations were determined by the center of gravity of the upper 5% (95% of band height) of the band. Bandwidth calculations were made at 70% of the band height. The algorithms for the above calculations are described in the literature [29]. The wavenumber positions of the CH_2 stretching vibrations of the 75% ethanol/H_2O-treated stratum corneum samples were determined following deconvolution of the 2960–2800 cm^{-1} spectral region. Deconvolution was necessary due to the interference of the ethanol methylene stretching vibrations in the region of the stratum corneum CH_2 stretching modes. The interferences would otherwise have compromised the center of gravity at 95% of band height calculations. Spectral deconvolution parameters were varied as needed (halfwidth: 8 to 20, K: 2 to 2.5). The stratum corneum CH_2 scissoring deformation vibrational band positions were determined from deconvolved spectra (halfwidth: 8, K: 2).

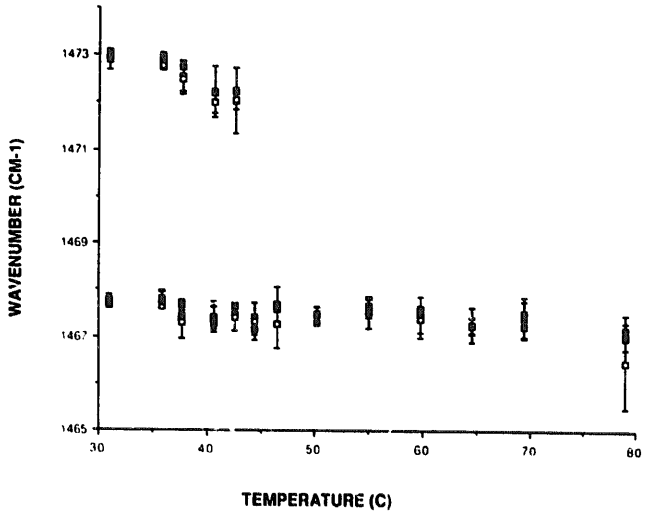

Fig. 1. Band position of the deconvolved (halfwidth = 8, K = 2) CH_2 scissoring deformation vibrations (1467 cm^{-1} band of doublet and 1472 cm^{-1} singlet) in excess D_2O (■), 20% perdeuterated ethanol/D_2O (□) and 36.7% perdeuterated ethanol/D_2O (△).

Results

The presence of a CH_2 scissoring doublet to singlet transition has been demonstrated in model lipid systems to result from a change in the gel phase packing of the lipid alkyl chains [30]. The transition is present in the stratum corneum lipid multilayers near 43°C (Fig. 1). An ethanol effect on the solid to solid phase transition and the gel phase packing of the stratum corneum lipid alkyl chains appears to be absent. This implies that the interchain interactions are similar in the three studies. Ethanol effects were also not observed with stratum corneum in the presence of 75% ethanol/water solutions.

Fig. 2 illustrates the CH_2 symmetric stretching bandwidth at 70% height as a function of temperature and ethanol concentration. The bandwidth is known to be sensitive to the freedom of vibrational and torsional motion of the lipid methylene groups within their immediate environments [31]. The mobility of the stratum corneum lipid alkyl chain methylene groups is slightly enhanced above the solid-solid phase transition (~ 43°C). Although the data are erratic due to inter-mouse variation, the transition region between 50 and 70°C appears to demonstrate a concentration dependent increase in bandwidth. Analysis of the regression coefficients (slope and y-intercept) of the linear portion of the transition regions of the three treatments indicates that there are significant differences in the y-intercepts of the three lines at the 95% confidence level. There are no significant differences between the slopes. There

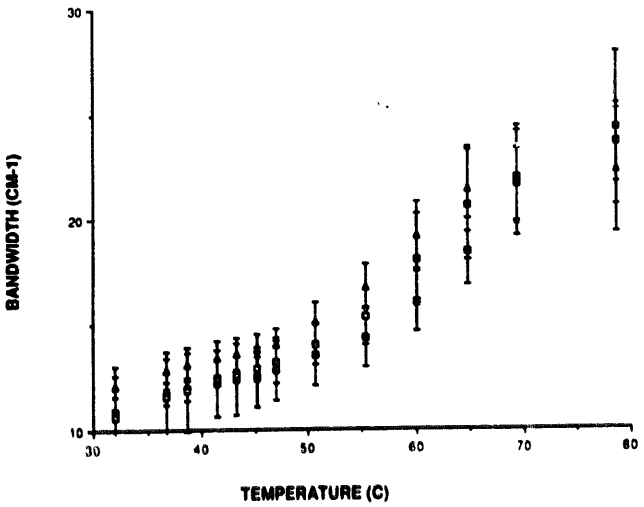

Fig. 2. CH_2 symmetric stretching bandwidth (cm^{-1}) at 70% of band height as a function of temperature in excess D_2O (■), 20% perdeuterated ethanol/D_2O (□) and 36.7% perdeuterated ethanol/D_2O (△).

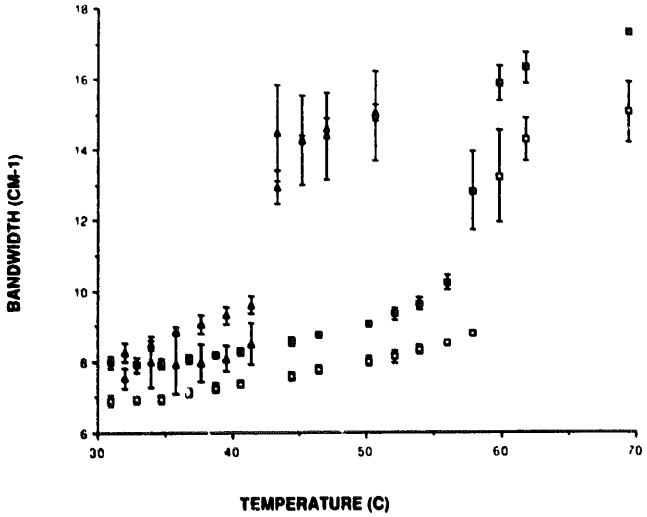

Fig. 4. CH_2 symmetric stretching bandwidth (cm^{-1}) at 70% band height as a function of temperature for DPPC/DPPA MLV in 100% D_2O (■), DPPC/DPPA MLV in 30% perdeuterated ethanol/D_2O (□), DSPC/DSPA MLV in 100% D_2O (△) and DSPC/DSPA MLV in 30% perdeuterated ethanol/D_2O (△).

appears to be no observable ethanol effect on the CH_2 symmetric stretching bandwidths below 55°C.

The stratum corneum CH_2 symmetric stretching band wavenumber positions as a function of temperature in the presence of 0, 20 and 36.7% perdeuterated ethanol/D_2O solutions are shown in Fig. 3. The vibrational band position reflects the ratio of *trans* to *gauche* conformers of a lipid alkyl chain [32]. An absorbance near 2848 cm^{-1} indicates a highly conformationally ordered (nearly all *trans*) chain [32], while a shift to higher wavenumbers reflects an increase in *gauche* conformer population [31]. No observable shift in band position is noted below 55°C in the presence of ethanol as compared to D_2O.

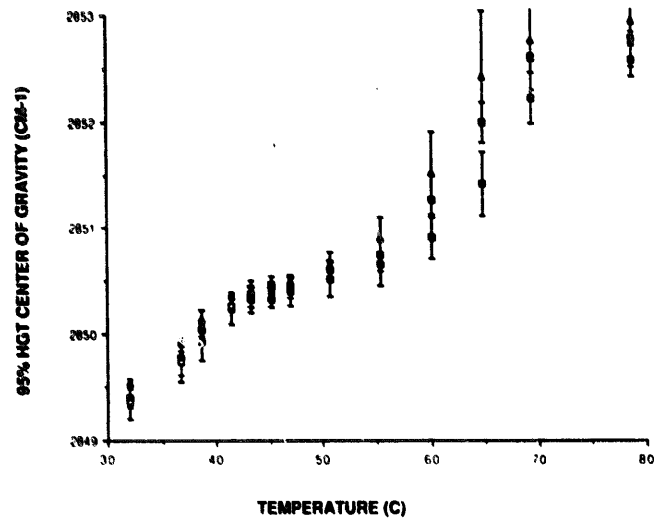

Fig. 3. CH_2 symmetric stretching band position (cm^{-1}) as determined by center of gravity at 95% of band height as a function of temperature in excess D_2O (■), 20% perdeuterated ethanol/D_2O (□) and 36.7% perdeuterated ethanol/D_2O (△).

A noticeable shift to lower temperatures in the gel to liquid-crystalline phase transition is observed above 55°C (Fig. 3). This reduction in the transition temperature is concentration dependent. A greater shift in the *trans* to *gauche* population is observed for the 36.7% solution at temperatures between 55 and 70°C. Analysis of the data at 65°C indicates that both ethanol concentrations induce a significant shift in band position compared to the 100% D_2O samples (unpaired Student's *t*-test, $P < 0.05$). A further reduction in the transition temperature was observed with stratum corneum in the presence of a 75% ethanol/water solution. Reductions in the gel to liquid-crystalline phase transition temperature of model phospholipid bilayer membranes in the presence of ethanol have also been reported with spectroscopic turbidity [21–23] and DSC [24] measurements.

Ethanol effects on the thermotropic phase behavior of model phospholipid MLV suspensions (DPPC/DPPA and DSPC/DSPA MLVs) have been investigated. Figs. 4 and 5 show the CH_2 symmetric stretching vibrational bandwidths at 70% height and wavenumber positions, respectively, as a function of temperature of the vesicle suspensions in 0 and 30% perdeuterated ethanol/MLV suspensions. Below the gel to liquid-crystalline transition at 41 and 58°C for DPPC/DPPA and DSPC/DSPA MLV suspensions, respectively (Fig. 4), a significant decrease in the bandwidth is observed indicating a decrease in chain mobility. This is also evident at temperatures throughout the transition and above. A shift of the band position (Fig. 5) to lower wavenumbers is observed below the gel to liquid-crystalline transition temperature with ethanol present indicating an increase in conformational order

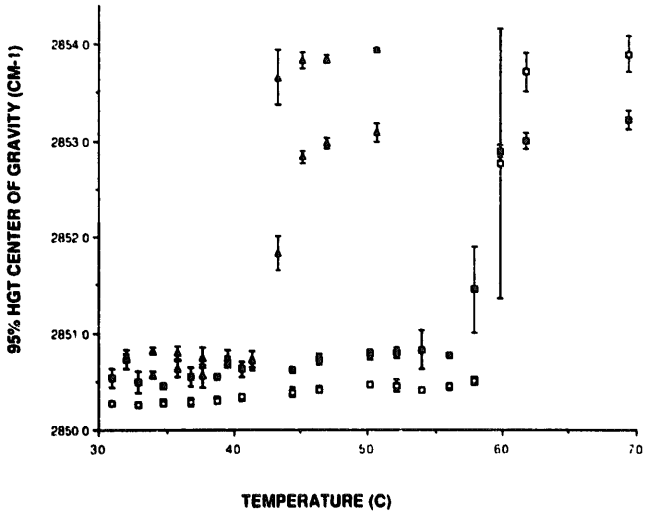

Fig. 5. CH_2 symmetric stretching band position (cm^{-1}) as determined by center of gravity at 95% band height as a function of temperature for DPPC/DPPA MLV in 100% D_2O (■), DPPC/DPPA MLV in 30% perdeuterated ethanol/D_2O (□), DSPC/DSPA MLV in 100% D_2O (▲) and DSPC/DSPA MLV in 30% perdeuterated ethanol/D_2O (△).

of the alkyl chains within the bilayer. Above the transition, a shift to higher wavenumbers is observed compared to the control, suggestive of an increase in chain conformational disorder.

The precision with which both the CH_2 symmetric stretching bandwidth at 70% height and center of gravity at 95% height are measured in the model phospholipid vesicle systems should be noted. It is apparent that the variability observed in these same measurements in stratum corneum can be attributed to mouse to mouse variation. Therefore, individual stratum corneum samples were studied before and after ethanol treatment. This procedure eliminates the effect of inter-mouse variability, as well as site to site differences within the same animal. The differences in

TABLE I

Reuse experiment with D_2O to perdeuterated ethanol / D_2O exchange

Sample	CH_2 asymmetric stretching		CH_2 symmetric stretching	
	ΔCG [a]	ΔBW [b]	ΔCG [a]	ΔBW [b]
1	+0.10	−1.36	+0.13	−0.86
2	+0.45	−3.59	+0.08	−1.45
3	+0.04	−1.54	+0.01	−0.67
4	−0.15	−1.80	−0.06	−1.68
5	+0.06	−1.67	+0.10	−0.93
6	0.00	−1.17	+0.01	−0.77

[a] ΔCG = Band position in presence of perdeuterated ethanol/D_2O–Band position in presence of D_2O solution.

[b] ΔBW = Bandwidth in presence of perdeuterated ethanol/D_2O–Bandwidth in presence of D_2O solution.

TABLE II

Reuse experiment with D_2O to D_2O exchange

Sample	CH_2 asymmetric stretching		CH_2 symmetric stretching	
	ΔCG [a]	ΔBW [b]	ΔCG [a]	ΔBW [b]
1	+0.07	−1.68	−0.03	+0.02
2	+0.12	+0.36	−0.04	+1.23
3	−0.13	+0.66	+0.07	+1.54

[a] ΔCG = Band position in presence of replacement D_2O–Band position in original D_2O solution.

[b] ΔBW = Bandwidth in presence of replacement D_2O–Bandwidth in original D_2O solution.

center of gravity at 95% band height (ΔCG) and bandwidth at 70% height (ΔBW) for both the CH_2 asymmetric and symmetric stretching vibrations for six samples before and after treatment with 30% perdeuterated ethanol/D_2O are listed in Table I. The differences in band position (ΔCG) appear to oscillate about zero indicating there are no significant shifts in band position of either the asymmetric or symmetric band in the presence of ethanol. However, the bandwidth differences are negative demonstrating a significant (nonparametric Sign test, $P < 0.05$) narrowing of both the CH_2 stretching vibrational bands in the presence of ethanol. The results of control experiments with stratum corneum samples treated identically as in the membrane reuse experiments described above are shown in Table II. The replacement solvent was 100% D_2O instead of 30% perdeuterated ethanol/D_2O. There is a fluctuation about 0 for both the CH_2 asymmetric and symmetric stretching band positions. However, the bandwidth differences are not negative as was observed in the ethanol treatments (Table I). Most samples gave a positive increase in bandwidth after the second treatment with 100% D_2O.

Discussion

It is evident from the current infrared data that ethanol does not significantly alter the degree of stratum corneum lipid inter-alkyl chain interactions (Fig. 1). The chains remain densely packed below 43°C with strong interchain coupling. There appear to be minimal effects on the less densely packed gel phase (Fig. 2) above 43°C. The mobility of the lipid methylene functional groups is slightly enhanced in the presence of low ethanol concentrations. Increased chain mobility may be related to the reduction in the gel to liquid-crystalline phase transition temperature (Fig. 3). The shift in the phase transition temperature has been observed with model phospholipid vesicle membranes in the presence of ethanol [21,22,24].

Rowe [21,22] has suggested that ethanol preferentially acts on the liquid crystalline regions of the lipid bilayers. This is consistent with spin label [11–16] and fluorescent probe [17–19] measurements of liquid-crystalline phase lipid membranes where ethanol has been demonstrated to fluidize the alkyl chains. The DSPC/DSPA and DPPC/DPPA vesicles show a significant decrease in conformational order (Fig. 5) above the gel to liquid-crystalline phase transition demonstrating ethanol interaction with the fluid lipids. It is suggested by Rowe [21,22] that the presence of ethanol results in a freezing point depression of the lipid alkyl chains. Jain and Wu [24] have argued that the interpretation of freezing point depression resulting in the shift in the transition temperature is not valid. Two assumptions are violated: (1) equimolar concentrations of different solutes give equal effects on the transition, and (2) lipid bilayers and biomembranes can be approximated by bulk solvent properties. It should be noted that further reduction in the lipid freezing point should result with increasing ethanol concentrations. However, an increase in the transition temperature of DPPC bilayers has been observed with high ethanol concentrations [21,22].

Hitzeman and co-workers [33,34] have demonstrated the high degree of ethanol partitioning into the headgroup region of DPPC bilayers and rat neuronal membranes. The presence of ethanol in the polar headgroup region of the lipid bilayers had previously been suggested by Jain and Wu [24] and Hui and Barton [35]. Ethanol may alter the polar headgroup intermolecular interactions due to its presence within the polar headgroup plane, thus reducing the energy required to induce the phase transition resulting in the observed reduction in the gel to liquid-crystalline phase transition temperature. However, Nagle [36] has suggested that the polar headgroup interaction energy plays a minimal role in the phase transition. Substitution of polar headgroups shifts the transition temperature by as little as 20°C. The reduction in the transition temperature of the stratum corneum in the presence of ethanol (Fig. 3) is slight (< 10°C) and could be accounted for by an alteration in the lipid polar headgroup interactions.

Many investigations have focused on the interactions of short chain alcohols, such as ethanol, on the liquid-crystalline phase of model and biomembranes [11–19,33,34]. However, the stratum corneum lipid multilayers at physiological temperature (37°C) are shown to be densely packed and highly conformationally ordered [26]. The effects of ethanol on the gel phase of lipid bilayers can be quite different from that in the liquid-crystalline phase. This is evident from the DSPC/DSPA and DPPC/DPPA vesicle studies (Fig. 5). Simon and McIntosh [25] have demonstrated that ethanol can induce an interdigitated gel phase in DPPC bilayers believed to result from intercalation of ethanol between lipid molecules in the polar headgroup region. A requirement for interdigitation to occur is that the chains must become more highly ordered to enable insertion between adjacent chains [25]. The DPPC/DPPA data are consistent with the X-ray data which also demonstrated increased order. It is possible that ethanol may be inducing an interdigitated gel phase in the DSPC/DSPA systems. It is reasonable to assume that the increased conformational order in the interdigitated gel phase could also be characterized by decreased alkyl chain mobility. This phenomenon is evident in both the DSPC/DSPA and DPPC/DPPA vesicle suspensions (Fig. 4).

The variability observed in Figs. 2 and 3 from averaged stratum corneum samples gives the misleading conclusion that the stratum corneum lipid alkyl chains are not significantly affected by the presence of ethanol below the gel to liquid-crystalline phase transition. A large number of stratum corneum studies would be required to suggest a statistically significant difference in the treatments under these conditions. However, by studying each sample before and after treatment with ethanol, significant differences can be measured with a reasonable number of stratum corneum samples (Table I). It is clear that the CH_2 asymmetric and symmetric stretching bandwidths decrease in the gel phase at 29°C with ethanol present. This effect is not evident in the control samples (Table II) which demonstrates that the reduced bandwidth (Table I) must be a direct effect of ethanol on the stratum corneum lipids. However, there is no corresponding increase in conformational order.

There are fundamental differences between stratum corneum lipids and the DSPC/DSPA and DPPC/DPPA vesicles which preclude their ability to function as perfect model systems for stratum corneum lipids. However, the information obtained from these membranes may provide qualitative insight into more complex membranes such as the stratum corneum. The magnitude of the decreased chain mobility in the presence of ethanol suggests that a limited population of the stratum corneum lipid alkyl chains may exist in the interdigitated gel state based on the X-ray investigations of Simon and McIntosh [25] for DPPC bilayers and the current FTIR vesicle data. The absence of increased conformational order may result from the fact that these chains are naturally highly ordered and a further increase in order is not necessary for the induction of limited interdigitated phases.

A preliminary X-ray diffraction experiment with hairless mouse stratum corneum under similar conditions was inconclusive in demonstrating the presence of an interdigitated gel phase (Stephen White, personal communication). However, the experiment could not be performed with the identical piece of stratum

corneum in the absence and presence of ethanol. Thus, inter-animal variation may have inhibited the ability to note differences.

It is apparent from the data that the stratum corneum lipid alkyl chains are not in predominantly the liquid-crystalline state in the presence of ethanol at physiologic temperature. There is no increase in lipid chain disorder nor increased chain mobility as would be expected of a 'fluidizing agent'.

The relationship between enhanced permeation of lipophilic solutes such as β-estradiol and hydrocortisone through hairless mouse skin in the presence of ethanol [9] is complex. Although the lipid alkyl chains are highly ordered and densely packed, the region of the bilayer that may possess the greatest resistance to diffusion is the polar head plane. Raykar [37] has suggested the relevance of the polar headgroup region to transbilayer passive diffusion. If the stratum corneum lipids are separated by the replacement of water with ethanol, an increase in the interfacial area of the lipid bilayers results. The intercalation of ethanol into the polar headgroup environment could disrupt the lipid intermolecular hydrogen bonding. Pascher [38] states that the reduction of interchain hydrogen bonds between ceramide lipids should result in an increase in the permeability of such membranes. Elias [39] and Wertz et al. [40] have demonstrated that the mouse and human stratum corneum has an abundance of ceramide lipids present. A significant amount of 24 and 26 carbon ω-hydroxyacid ceramide lipids has been identified in human stratum corneum [40]. This class of ceramides possesses interlipid hydrogen bonding capabilities. However, the ethanol-induced increased order within the stratum corneum as evidenced by the bandwidth narrowing does not suggest involvement of a significant population of stratum corneum lipids.

Veiro et al. [42] have recently studied the effects of n-alkanols on the pretransition of DPPC bilayers. It is suggested that ethanol may decrease the energy required to form the bilayer ripple ($P_{\beta'}$) phase from the lamellar ($L_{\beta'}$) phase. In the $P_{\beta'}$ phase a high degree of hydrocarbon is exposed to the aqueous interface. This would result in a decrease in the interchain polar headgroup interactions which correlates well with the loss of the barrier properties of a bilayer due to a disruption of the polar headgroup environment.

Rowe [43] has investigated the thermotropic phase behavior of mixed phosphatidylethanolamine (PE) and phosphatidylcholine (PC) lipids in the presence of ethanol. The question is raised if both an interdigitated and non-interdigitated gel phase can exist in the same lamella. Boggs et al. [44] have suggested based on ESR determinations of the extent of polymixin B induced interdigitation in mixed lipid systems that phase separation occurs between the noninterdigitated and interdigitated lipids. Based on the current infrared data, the possible existence of limited interdigitated domains can not be eliminated. If limited interdigitated domains exist in the stratum corneum, an increase in solute permeation through these regions would be expected as a result of decreased polar headgroup interactions or at the interface between phases. However, the ability of ethanol to induce increased order, suggesting interdigitation, of stratum corneum lipids must be investigated more thoroughly in relevant model systems.

Under ordinary conditions, a reduction in the diffusional distance across a membrane would result in enhanced permeation. However, in a densely packed, interdigitated phase the decrease in methylene group mobility would suggest a greater diffusional resistance per methylene group than in a noninterdigitated phase. Therefore, the reduced thickness of bilayers alone would not be expected to exhibit increased permeability. Other mechanisms must be considered to explain ethanol-induced enhancement through the stratum corneum lipid multilayers. It has been shown in this study that ethanol disorders phospholipid vesicles in the liquid-crystalline state, although increased alkyl chain disorder and freedom of motion were not detected in the current FTIR studies of ethanol-induced effects on the stratum corneum at low concentrations.

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References

1 Blank, I.H., Scheuplein, R.J. and McFarland, D.J. (1967) J. Invest. Dermatol. 49, 582–589.
2 Behl, C.R., Barrett, M., Flynn, G.L., Kurihara, T., Walters, K.A., Gatmaitan, O.G., Harper, N., Higuchi, W.I., Ho, N.F.H. and Pierson, C.L. (1982) J. Pharm. Sci. 71, 229–234.
3 Michaels, A.S., Chandrasekaran, S.K. and Shaw, J.E. (1975) Am. Inst. Chem. Eng. J. 21, 985–996.
4 Knutson, K., Potts, R.O., Guzek, D.B., Golden, G.M., McKie, J.E., Lambert, W.J. and Higuchi, W.I. (1985) J. Controlled Release 2, 67–87.
5 Knutson, K., Krill, S.L., Lambert, W.J. and Higuchi, W.I. (1987) J. Controlled Release 6, 59–74.
6 Golden, G.M., McKie, J.E. and Potts, R.O. (1987) J. Pharm. Sci. 76, 25–28.
7 Kurihara-Bergstrom, T., Flynn, G.L. and Higuchi, W.I. (1986) J. Pharm. Sci. 75, 479–486.
8 Lambert, W.J. Ph.D. Dissertation, University of Utah (1987).
9 Ghanem, A.H., Mahmoud, H., Higuchi, W.I., Rohr, U.D., Borsadia, S., Liu, P., Fox, J.L. and Good, W.R. (1987) J. Controlled Release 6, 75–83.

10 Tillotson, L.G., Carter, E.A., Inui, K.I. and Isselbacher, K.J. (1981) Arch. Biochem. Biophys. 207, 360–370.
11 Fernandez, Y.J., Boigegrain, R.-A.M., Cambon-Gros, C.D. and Mitjavila, S.E. (1984) Biochim. Biophys. Acta 770, 171–177.
12 Sauerheber, R.D., Esgate, J.A. and Kuhn, C.E. (1982) Biochim. Biophys. Acta 691, 115–124.
13 Lenaz, G., Bertoli, E., Curatola, G., Mazzanti, L. and Bigi, A. (1976) Arch. Biochem. Biophys. 172, 278–288.
14 Pang, K.Y.Y., Braswell, L.M., Chang, L., Sommer, T.J. and Miller, K.W. (1980) Mol. Pharmacol. 18, 84–90.
15 Lyon, R.C., McCombe, J.A., Schreurs, J. and Goldstein, D.B. (1981) J. Pharmacol. Exp. Ther. 218, 669–675.
16 Chin, J.H. and Goldstein, D.B. (1977) Mol. Pharmacol. 13, 435–441.
17 Johnson, D.A., Lee, N.M., Cooke, R. and Loh, H.H. (1979) Mol. Pharmacol. 15, 739–746.
18 Harris, R.A., Burnett, R., McQuilkin, S., McClard, A. and Simon, F.R. (1987) Ann. N.Y. Acad. Sci. 492, 125–135.
19 Harris, R.A. and Schroeder, F. (1982) J. Pharmacol. Exp. Ther. 223, 424–431.
20 Harris, R.A. and Schroeder, F. (1981) Mol. Pharmacol. 20, 128–137.
21 Rowe, E.S. (1983) Biochemistry 22, 2999–3305.
22 Rowe, E.S. (1982) Mol. Pharmacol. 22, 133–139.
23 Rowe, E.S. (1985) Biochim. Biophys. Acta 813, 321–330.
24 Jain, M.K. and Wu, N.M. (1977) J. Membr. Biol. 34, 157–201.
25 Simon, S.A. and McIntosh, T.J. (1984) Biochim. Biophys. Acta 773, 169–172.
26 Krill, S.L., Knutson, H. and Higuchi, W.I. (1992) Biochim. Biophys. Acta 1112, 281–286.
27 Bummer, P.M. (1987) Ph.D. Dissertation, University of Wisconsin-Madison.
28 Forte, T.M. and Nordhausen, R.W. (1986) Methods Enzymol. 128, 442–457.
29 Cameron, D.G., Kauppinen, J.K., Moffatt, D.J. and Mantsch, H.H. (1982) Appl. Spectrosc. 36, 245–250.
30 Cameron, D.G., Casal, H.L., Gudgin, E.F. and Mantsch, H.H. (1980) Biochim. Biophys. Acta 596, 463–467.
31 Casal, H.L., Cameron, D.G., Smith, I.C.P. and Mantsch, H.H. (1980) Biochemistry 19, 444–451.
32 Snyder, R.G., Strauss, H.L. and Elliger, C.A. (1982) J. Phys. Chem. 86, 5145–5150.
33 Hitzeman, R.J., Graham-Brittain, C., Schueler, H.E. and Kreishman, G.P. (1987) Ann. N.Y. Acad. Sci. 492, 142–144.
34 Hitzemann, R.J., Schueler, H.E., Graham-Brittain, C. and Kreishman, G.P. (1986) Biochim. Biophys. Acta 859, 189–197.
35 Hui, F.K. and Barton, P.G. (1973) Biochim. Biophys. Acta 296, 510–517.
36 Nagle, J.F. (1980) Annu. Rev. Phys. Chem. 31, 157–195.
37 Raykar, P.V. (1988) Ph.D. Dissertation, The University of Utah.
38 Pasher, I. (1976) Biochim. Biophys. Acta 455, 433–451.
39 Elias, P.M. (1981) J. Invest. Dermatol. 20, 1–19.
40 Wertz, P.W., Swartzendruber, D.C., Madison, K.C. and Downing, D.T. (1987) J. Invest. Dermatol. 89, 419–425.
41 Long, S.A., Wertz, P.W., Strauss, J.S. and Downing, D.T. (1985) Arch. Dermatol. Res. 277, 284–287.
42 Veiro, J.A., Nambi, P., Herold, L.L. and Rowe, E.S. (1987) Biochim. Biophys. Acta 900, 230–238.
43 Rowe, E.S. (1987) Biochemistry 26, 46–51.
44 Boggs, J.M., Wang, H.Y., Rangaraj, G. and Tummler, B. (1989) Biochim. Biophys. Acta 985, 199–210.