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Biochimica et Biophysica Acta 1195 (1994) 169–179

BBA Biochimica et Biophysica Acta

Enhanced permeation of polar compounds through human epidermis. I. Permeability and membrane structural changes in the presence of short chain alcohols

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Received 12 August 1993

Abstract

The influence of alcohol chain length on polar compound permeation in human skin was investigated to further understand alcohol-enhanced permeation mechanisms. Both thermodynamic and kinetic variables associated with the enhanced permeation of mannitol were ascertained in the presence of high concentrations of short chain alcohols. Permeation of mannitol through human epidermis in the presence of 75% (v/v) alcohol-saline mixtures was determined in symmetric, side-by-side diffusion cells at 32°C. Permeability coefficients increased with increasing alcohol chain length (iso-propanol > ethanol > methanol). Uptake of mannitol into the epidermal tissue increased in the presence of the short chain alcohols, but was independent of alcohol chain length. In addition, mannitol solubility decreased in the presence of the short chain alcohols, but again was independent of alcohol chain length. Therefore, increased mannitol permeability with increasing alcohol chain length could not be attributed to thermodynamic variables. Changes in the amount and conformation of stratum corneum lipids and proteins were determined by Fourier transform infrared (FTIR) spectroscopy. Stratum corneum lipid conformation and mobility was not significantly altered in the presence of the short chain alcohols. However, decreased absorbance of the alkyl chain suggested lipid extraction, which increased with increasing alcohol chain length. Stratum corneum protein conformation was altered in the presence of the short chain alcohols. Decreased infrared absorbance of the Amide I band maximum suggested extraction of stratum corneum proteins, which increased with increased alcohol chain length. These results suggest a correlation between enhanced permeation and extraction of lipids as well as proteins from human skin in the presence of 75% (v/v) aqueous alcohol solutions.

Keywords: Stratum corneum; Percutaneous absorption; Alcohol; Polar compound

1. Introduction

To improve percutaneous absorption, therapeutic solutes are formulated in combination with solvents or compounds, frequently referred to as penetration enhancers, which serve to increase solute transport across the outer, rate-limiting, heterogeneous stratum corneum layer of the skin. The stratum corneum is composed primarily of keratinized cells embedded in a lipid-rich multilamellar matrix [1], which shields the body against desiccation and environmental assault. Transport of polar solutes across the stratum corneum layer may be enhanced through an increase in solute solubility within the formulation, providing a greater driving force for compound flux, and/or through alteration of stratum corneum structure or composition, which results in an increase in membrane permeability. Short chain alcohols, such as ethanol and iso-propanol, are common components of transdermal systems and topical formulations [2–4]. It is important to gain an understanding of the possible mechanisms by which short chain alcohols act as penetration enhancers for polar solutes.

Various aspects of alcohol-enhanced transdermal drug delivery have been studied through experiments performed in the presence of as well as after treatment with short chain alcohols [2–11]. The ability of short chain alcohols to enhance polar solute permeation increases with increasing alcohol concentration [8–11]. Ethanol both increases percutaneous absorption of po-

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SSDI 0005-2736(94)00158-L

lar solutes and removes intercellular lipids through extraction [11]. The influences of short chain alcohols on the rate-limiting stratum corneum membrane have been explored directly at low concentrations [12,13]; however, no information is available regarding their effects at high concentratons. For model lipid systems, alcohol effects on lipid biophysical structure are biphasic and concentration-dependent [14,15]. Therefore, it is essential that alcohol effects on stratum corneum lipids be studied at high concentrations in order to determine possible contributions to enhanced transport of polar solutes. In addition, alcohols swell keratin proteins [16,17]; hence, alcohol effects on stratum corneum proteins as well as the lipid matrix must be determined to also understand possible contributions of protein structural alterations to enhanced permeation.

The mechanism(s) of alcohol-enhanced polar solute permeation through skin may involve both membrane-associated variables (conformational alterations within and extraction of membrane components) and solute-associated variables (partitioning and solubility). The objective is to determine the alcohol-related changes in both membrane- and solute-associated variables in the presence of the short chain alcohols.

The influence of increasing alcohol carbon number on the permeation of the hydrophilic compound mannitol through human epidermis was determined in solutions of 75% (v/v) alcohol in saline. Thermodynamic parameters associated with solute permeation, such as stratum corneum-to-vehicle partitioning and mannitol activity in the donor solutions [18] were also determined in the presence of the aqueous alcohol solutions. Alterations of stratum corneum biophysical structure in the presence of the aqueous alcohols were determined using Fourier transform infrared spectroscopy (FTIR). The applicability of FTIR for study of the stratum corneum membrane has been proven under a variety of conditions [11,12,19–23]. Thus, insight into the mechanism(s) of alcohol-enhanced permeation for polar compounds can be gained by understanding the contributions of alcohol-induced changes in thermodynamic parameters as well as stratum corneum biophysical structure to increased mannitol permeation.

2. Materials and methods

The short chain alcohols used in these studies include: methanol (HPLC grade, J.T. Baker, Phillipsburg, NJ), ethanol (absolute, USP grade, Midwest Grain Products, Weston, MO), and 2-propanol (HPLC grade, Burdick & Jackson, Muskegon, MI). Gentamicin sulfate solution (0.1%, Sigma) was added to each diffusion solution (to a final concentration of 0.01%) to inhibit bacterial growth. Radiolabeled mannitol (D-[1(n)-^{3}H]mannitol, 19.1 Ci/mmol, New England Nuclear, Boston, MA) was employed as the diffusing solute.

2.1. Skin preparation

Skin bank human skin, female abdomen and back, was donated by Ciba-Geigy. The skin bank skin, stored frozen at −70°C, was thawed in 25°C saline 1–2 h prior to use. Human epidermis was isolated from the underlying dermis by heat separation in 60°C saline for 2.0 min, leaving the stratum corneum and epidermal layers intact [24]. Stratum corneum sheets were isolated from heat-separated human epidermis by incubation in a 0.5% solution of trypsin (from porcine pancreas, Type IX Sigma, St. Louis, MO) in PBS, pH 8.0, for 4 h at 37°C. After rinsing the stratum corneum sheets with distilled water to remove excess epidermal cells and trypsin, the sheets were vacuum dried (10^{-4} Torr) at room temperature and stored desiccated over Drierite (Aldrich, Milwaukee, WI).

2.2. Permeation experiments

Diffusion of the polar compound mannitol through human epidermis from several skin donors was determined in saline and in 75% (v/v) alcohol/saline mixtures. Solutions of 75% (v/v) methanol, ethanol, and 2-propanol in saline were employed to achieve enhanced permeation while maintaining a constant volume fraction alcohol. Equivalent volume fractions of alcohol, although pharmaceutically relevant, do not represent equivalent levels of alcohol activity within the 75% aqueous alcohol solutions. Experiments were performed in symmetric, horizontal diffusion cells at 32°C. The epidermal membranes were mounted between the half cells with the stratum corneum facing the donor chamber. Donor and receiver chambers were both filled with 2.0 ml of either saline or 75% (v/v) methanol, ethanol, or 2-propanol/saline mixtures (with 0.1% gentamicin sulfate solution, Sigma Chemical Co., to inhibit bacterial growth) and spiked at time zero with 10–30 μCi mannitol (D-[1(n)-^{3}H]mannitol, 19.1 Ci/mmol, New England Nuclear, Boston, MA). Samples were withdrawn from the receiver chambers every 3–12 h over a 50 h period. The receiver volume was replenished with fresh solution after each sample was taken. Donor chamber samples (50 μl) were taken periodically throughout the experiment without replacement. Mannitol concentrations were determined by liquid scintillation counting (LS 1801, Beckman Instruments, Fullerton, CA).

Permeability coefficients, K_P, were calculated using the equation:

$$K_P = V_R(dC_R/dt)/A\Delta C \quad (1)$$

where V_R is the receiver chamber volume, dC_R/dt is the change in receiver concentration with time, A is the diffusional area of the cell (average = 0.679 cm^2) and ΔC is the donor-to-receiver concentration gradient. Linear regression of receiver sample data collected after attainment of steady-state flux was used to calculate dC_R/dt. Lag times were calculated from the x-intercept of dC_R/dt. The dilution effect of sample replacement in the receiver chamber was corrected for in the calculations.

2.3. Stratum corneum uptake experiments

Stratum corneum disks punched from whole stratum corneum sheets with a cork borer (1.3 cm diameter) were hydrated 1 h in saline or 75% (v/v) alcohol/saline mixtures before measuring thickness and diameter. Stratum corneum volume was calculated from hydrated stratum corneum thickness, measured with a lightwave micrometer (1.0 μm accuracy, Van Keun, Waterford, MA), and surface area of the disk, determined from the average disk diameter, measured to the nearest 0.1 cm. The disks were then incubated 48 h at 32°C in 3 ml aqueous alcohol solution (containing 0.01% (w/v) gentamicin sulfate) spiked with 5 μl [^{3}H]mannitol. After equilibration, stratum corneum disks were removed from solution, blotted dry, rinsed and blotted dry twice in fresh water or 75% alcohol/water, and vacuum dried 24–48 h. The samples were then digested with Soluene 350 tissue solubilizer, 100 μl/mg tissue, and submitted for liquid scintillation counting (Beckman LS 1800, Fullerton, CA).

Stratum corneum uptake of mannitol was determined by calculation of apparent partition coefficients, K_m, according to the equation:

$$K_m = C_{\text{Stratum Corneum}}/C_{\text{Donor Solution}} \quad (2)$$

where concentrations are given in mmol/ml. Solute concentration in the stratum corneum was determined by dividing the amount of radiolabeled drug in the stratum corneum by the hydrated stratum corneum volume. The average of 4–8 experiments (4 or 5 skin sources) is reported for each alcohol solution.

2.4. Mannitol solubility

Solubility of mannitol in water and 75% alcohol/water solutions was determined gravimetrically. An excess of unlabeled mannitol (Sigma, St. Louis, MO) was added to 12.5 ml of solvent and stirred 48 h at room temperature to prepare saturated solutions. After allowing each solution to settle undisturbed for 72 h, 5 ml aliquots of supernatant were removed, evaporated to dryness under a stream of dry nitrogen, and vacuum dried (10^{-4} Torr, 16 h, 25°C) to remove all solvent. The weight of residual mannitol from each supernatant aliquot was determined to the nearest 10 μg on a Mettler M3 Microbalance (Mettler, Highstown, NJ). Mannitol solubility was calculated as the weight of residual mannitol divided by the aliquot volume (μg mannitol per ml solution).

2.5. Stratum corneum biophysical structure

Alcohol-induced changes in stratum corneum biophysical structure were determined through FTIR solvent replacement experiments [12]. Infrared spectra were obtained under isothermal conditions in the presence of deuterium oxide (D_2O, 99.9% D, Cambridge Isotope Laboratories, Woburn, MA) and 75% (v/v) alcohol/D_2O solutions (perdeuterated, 99.8% D, Cambridge Isotope Laboratories) to simulate conditions previously studied in the permeation experiments. Stratum corneum samples were hydrated overnight in saturated D_2O vapor and then equilibrated in D_2O for 2–3 h. The sample was sealed in an excess of D_2O between two zinc selenide crystals and placed in a Harrick flow-through transmission sample holder (Harrick Scientific, Ossining, NY) equipped with two solvent ports and sealed. Spectra were obtained by an FTIR spectrometer (Digilab FTS 20/80, Bio-Rad, Cambridge, MA) equipped with a liquid nitrogen cooled mercury-cadmium-telluride narrow band (4000–800 cm^{-1}) detector at 2 cm^{-1} resolution (1024 scans, triangular apodization, 0.1 noise level and a zero fill factor of 4) under nitrogen purge. After obtaining an initial sample spectrum in D_2O, the D_2O in the sample cell was gradually replaced with 2.0 ml of fresh D_2O or 75% perdeuterated alcohol in D_2O over a period of 2.5 h. After solvent replacement, FTIR spectra of the stratum corneum were recorded hourly for up to 9 h. D_2O hydration of stratum corneum does not result in significant frequency shifts of the CH_2 C-H stretching bands [21]; therefore, D_2O solvent replacement will not indicate whether the sample has reached equilibrium with respect to hydration. However, solvent replacement with D_2O provides a baseline control to confirm that the sample does not shift out of position during solvent replacement.

The solvent-induced alterations in stratum corneum biophysical structure were monitored by following changes in band position, bandwidth, and band shape. Spectral manipulations were performed with SpectraCalc (Galactic Industries, Salem, NH) and Digilab data manipulation packages. Band position was determined by calculating the center of gravity (CG) of each band at 95% of band height and full bandwidth was determined at 70% of the band height [25]. Changes in lipid alkyl chain packing were determined by deconvolution (Digilab software, k-value = 2.0, halfwidth = 8.0, triangular apodization) of the CH_2 scissoring doublet/singlet, centered near 1460 cm^{-1}. Spectra were decon-

Table 1
Mannitol permeability coefficients lag times and solubilities in solutions of saline or 75% alcohol in saline

Solvent	$K_P \cdot 10^8$ (cm/s)	Lag time (min)	n/D	Solubility (μg/ml) $n = 4$
Saline	0.54 (0.75)	−60 (43)	9/2	185 (0.6)
75% Methanol	3.90 (1.20)	30 (28)	14/5	11.7 (0.05)
75% Ethanol	15.16 (7.93)	310 (211)	26/6	10.6 (0.03)
75% 2-Propanol	93.44 (54.5)	844 (250)	20/5	11.7 (0.05)

Reported as mean (S.D.). n/D = number of experiments/number of skin donors.

volved in the Amide I region, 1700–1550 cm^{-1} (Digilab software, k-value = 2.6, halfwidth = 20.0, triangular apodization), to determine conformational changes of stratum corneum proteins. Second derivative spectra were calculated using a bandedge of 0.1–0.15 (Digilab software) to confirm Amide I band positions determined in deconvolved spectra.

Further identification of conformational changes in stratum corneum lipids or proteins was accomplished by FTIR absorbance difference spectra [26]. Absorbance difference spectra were calculated for each solvent replacement experiment:

$$\text{Difference spectrum} = \text{Sample spectrum(75\% Alcohol)} - \text{Sample spectrum}(D_2O) \quad (3)$$

Negative bands in an absorbance difference spectrum indicate a decrease in the conformational population of a given molecular group as a result of exposure to the alcohol solution, whereas positive bands indicate an increase in the population. Each sample serves as its own control, thereby minimizing variability and providing maximum sensitivity for detection of minor structural alterations. As solvent is flushed through the transmission cell, microbubbles often form, altering the index of refraction along the infrared beam path and giving rise to a periodic sine wave, an artifact of the experimental protocol. This sine wave limits the sensitivity of absorbance difference spectra for weakly-absorbing bands.

Absorbance or band intensity is proportional to mol fraction (concentration) and molar absorptivity of the molecular group in a particular conformation or environment. The extent of alcohol-induced conformational changes and component extraction were quantified by determining relative changes in band absorbance (Abs) after solvent replacement:

$$\Delta Abs/Abs = (Abs_{\text{Alcohol}/D_2O} - Abs_{D_2O})/Abs_{D_2O} \quad (4)$$

Increasing absolute values for $\Delta Abs/Abs$ at a given wavelength indicate increased perturbation of the molecular vibrational modes giving rise to that band.

3. Results

3.1. Mannitol permeation

Representative plots of mannitol flux as a function of time are given in Fig. 1. The time required to achieve an apparent steady-state mannitol flux was determined through calculation of lag times. The effective lag times increased significantly with increasing

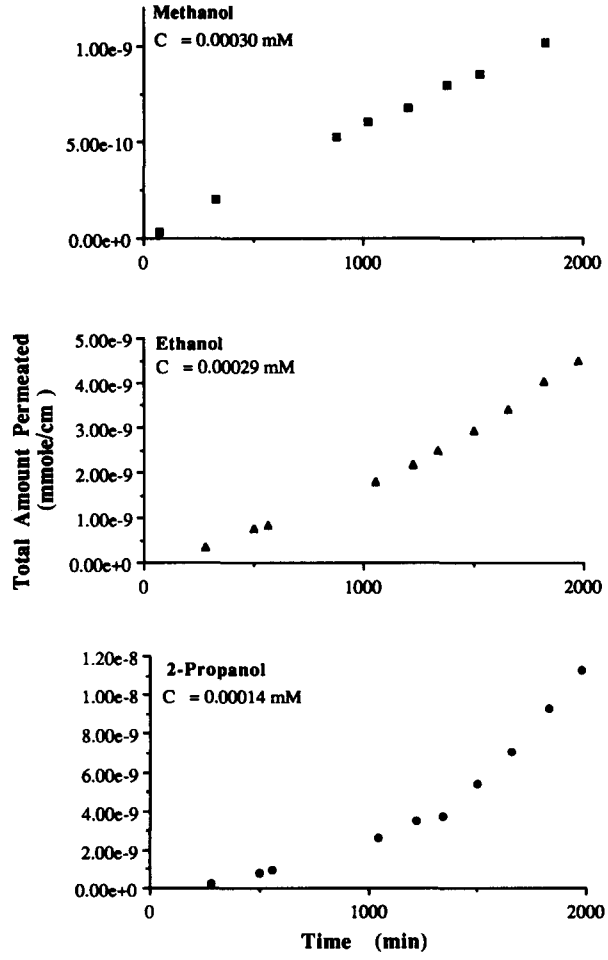

Fig. 1. Representative plots of mannitol flux (total amount permeated versus time) through human epidermis in the presence of 75% alcohol/saline solutions.

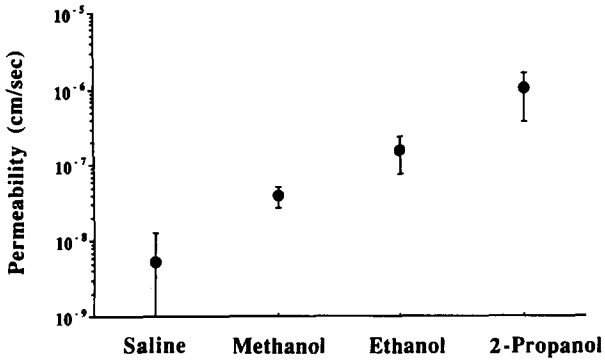

Fig. 2. Mannitol permeability coefficients through human epidermis as a function of alcohol chain length. Alcohol solutions prepared as 75% alcohol in saline.

alcohol chain length (Table 1). The increase in time required to attain steady state with increasing alcohol chain length suggests increased mannitol interaction(s) with the stratum corneum membrane as alcohol lipophilicity increases. Overall, mannitol permeability coefficients in human skin increased log-linearly with increasing alcohol carbon number in the presence of 75% (v/v) methanol, ethanol, and 2-propanol in saline (Fig. 2, Table 1).

3.2. Stratum corneum uptake

Uptake of mannitol into stratum corneum, calculated as apparent partition coefficients, K_m, increased from saline to the 75% alcohol/saline mixtures. However, stratum corneum uptake was not dependent on alcohol carbon number (Table 2), i.e., mannitol uptake into stratum corneum from solutions of 75% methanol, ethanol, or 2-propanol in saline was similar ($P > 0.05$). In determining uptake, stratum corneum thickness was also measured. The membrane thickness in the presence of short chain alcohols gradually decreased with increasing alcohol chain length. This decreased thickness has also been noted in histological preparations of human stratum corneum after treatment with solutions of 95% ethanol (L.K. Pershing, University of Utah, personal communication).

Table 2
Mannitol uptake into stratum corneum from saline or 75% alcohol/saline solutions

Solvent	Apparent partitioning (mM)/(mM)	Thickness (μm)	n/D
Saline	0.32 (0.04)	21.7 (4.4)	4/4
75% Methanol	0.96 (0.14)	21.2 (4.4)	8/7
75% Ethanol	0.79 (0.13)	18.6 (4.3)	8/7
75% 2-Propanol	0.82 (0.25)	15.0 (2.4)	5/5

Changes in stratum corneum thickness in the presence of 75% alcohols are also reported. Values reported as mean (S.D.). n/D = number of samples/number of skin donors.

3.3. Mannitol solubility in 75% alcohols

Mannitol solubility decreased from 185 ($\pm$0.60) μg/ml in water to 11.7 ($\pm$0.05) μg/ml in 75% alcohol/water mixtures (Table 1). As with stratum corneum uptake, mannitol solubility was equivalent ($P > 0.1$) for each of the 75% alcohol/water solutions: 11.7 ($\pm$0.05), 10.6 ($\pm$0.03), and 11.7 ($\pm$0.05) μg/ml for 75% methanol, ethanol, and 2-propanol in water, respectively.

3.4. Stratum corneum biophysical structure

For polar/hydrophilic solutes, the stratum corneum is the rate-limiting barrier to transport through skin [27,28]. Thus, an analysis of the effects of high concentrations of short chain alcohols directly on the stratum corneum sheets is essential for interpretation of alcohol-enhanced diffusion mechanisms. FTIR solvent replacement experiments enable the alcohol-induced changes in stratum corneum biophysical structure to be determined in the presence of aqueous alcohol solutions similar to those solutions used in the permeation experiments.

3.5. Stratum corneum lipids

For most lipid systems, the methylene group C-H asymmetric and symmetric stretching bands are sensitive to lipid alkyl chain conformation as well as torsional and librational mobility [29–35]. Packing of lipid alkyl chains may also be determined from methylene group C-H scissoring bands [30,36,37]. Therefore, stratum corneum lipid biophysical structure may be determined through analysis of the infrared bands arising from lipid alkyl chain methylene vibrational modes.

For model lipid systems, such as alkanes/paraffins [31], phospholipids [29,32], and cell membranes [33–35], alkyl chains consisting primarily of *trans* CH_2 conformers give rise to CH_2 C-H asymmetric and symmetric stretching bands near 2920 and 2850 cm^{-1}, respectively. Increasing the numbers of *gauche* conformers through thermal perturbation will shift these bands towards higher wavenumbers: 2924 and 2854 cm^{-1} for the CH_2 asymmetric and symmetric C-H stretching vibrations, respectively.

In untreated, D_2O-hydrated human stratum corneum, the average band positions (centers of gravity) were 2919 and 2850 cm^{-1} for the CH_2 C-H asymmetric and symmetric stretching bands, respectively, indicating that the majority of CH_2 conformers along the alkyl chains were *trans*, arising from stratum corneum lipids in a solid gel state. These band positions agree with published results for CH_2 stretching band positions of untreated stratum corneum [12,19–21,23,38]. The band positions did not shift ($P \leq 0.4$) after re-

Table 3
Changes in the CH_2 C-H stretching band positions (centers of gravity, 95% ht) after solvent replacement of D_2O with 75% alcohol/D_2O solutions

Solvent	CH_2 asymmetric ΔCG	CH_2 symmetric ΔCG	n
D_2O	0.016 (0.11)	−0.03 (0.12)	5
75% Methanol	−0.01 (0.10)	−0.06 (0.15)	8
75% Ethanol	−0.10 (0.05)	−0.01 (0.07)	8
75% 2-Propanol	−0.09 (0.10)	−0.03 (0.19)	9

Reported as mean ΔCG (S.D.). $\Delta CG = CG_{Alcohol/D_2O} - CG_{D_2O}$ for each sample.

placement of D_2O with 75% methanol/D_2O mixtures (Table 3). However, 75% ethanol and 2-propanol solvent replacement gave rise to a slight shift in the CH_2 asymmetric stretching band toward lower wavenumbers ($\Delta CG = -0.1$ cm^{-1}; $P < 0.05$). A shift in the CH_2 stretching band positions to lower wavenumbers would be associated with increased *trans* conformers along the alkyl chains. However, an average band shift towards lower wavenumbers of 0.1 cm^{-1}, although statistically significant, may not contribute significantly to changes in barrier function. For comparison, Krill et al. [12] have also reported that ethanol at 30% (v/v) concentration did not significantly shift the CH_2 band positions of hairless mouse stratum corneum lipids. Therefore, the higher concentrations of ethanol employed in these experiments exert a greater influence on stratum corneum lipid biophysical structure.

The CH_2 C-H stretching bandwidths (at 70% height) reflect the degree of heterogeneity (disorder) of C-H oscillators and their torsional and librational mobilities along the alkyl chains. Changes in bandwidth after solvent replacement with a given alcohol would therefore suggest alteration of methylene group environments along the lipid alkyl chains in the presence of the aqueous alcohol solution. The CH_2 stretching bandwidths remained unchanged or decreased in the control samples after D_2O solvent replacement with D_2O (Table 4). For 75% methanol/D_2O solvent replacement, the CH_2 stretching bandwidths remained unchanged ($P > 0.1$). For both 75% aqueous ethanol and 2-propanol, CH_2 asymmetric stretching bandwidths increased an average of 0.46 ($\pm$0.41) and 1.17 ($\pm$1.08) cm^{-1} ($P \leq 0.01$) and the CH_2 symmetric stretching bandwidths increased an average of 0.34 ($\pm$0.24) and 0.68 ($\pm$0.55) cm^{-1} ($P \leq 0.005$), respectively, after solvent replacement. This subtle increase in bandwidth suggests an increase in the heterogeneity (disorder) of the C-H oscillators along the lipid alkyl chains in the presence of 75% ethanol or 2-propanol. Such an altered environment along the alkyl chains could result from alcohol-promoted lipid extraction which may remove specific lipid conformations from the intercellular matrix. It is of interest that solutions of 30% (v/v) ethanol decrease the CH_2 bandwidths (average $\Delta BW = -1.51$ and -1.06 cm^{-1} for the CH_2 asymmetric and symmetric bands, respectively) of hairless mouse stratum corneum when similar solvent replacement methods were employed [12]. Therefore, ethanol effects on stratum corneum lipids appear to be concentration dependent, as has been noted for phospholipid systems [14,15].

Table 4
Changes in CH_2 C-H stretching bandwidths (70% ht) after solvent replacement of D_2O with 75% alcohol/D_2O solutions

Solvent	CH_2 asymmetric ΔBW	CH_2 symmetric ΔBW	n
D_2O	−0.32 (0.22)	−0.02 (0.13)	5
75% Methanol	0.36 (0.92)	0.15 (0.38)	8
75% Ethanol	0.46 (0.41)	0.34 (0.24)	8
75% 2-Propanol	1.17 (1.08)	0.68 (0.55)	9

Reported as mean ΔBW (S.D.). $\Delta BW = BW_{Alcohol/D_2O} - BW_{D_2O}$ for each sample.

Absorbance difference spectra (Eq. (3)) in the C-H stretching region (3000–2800 cm^{-1}) show specifically which conformational populations were altered in the presence of aqueous alcohol mixtures relative to D_2O, giving rise to the above changes in stratum corneum lipid band position and bandwidth. The initial CH_2 stretching band positions and bandwidths are indicative of ordered bilayers with alkyl chains primarily *trans* in conformation (Fig. 3A). Representative absorbance difference spectra (Eq. (3)) in the C-H stretching region for D_2O and 75% alcohol/D_2O solvent replacement experiments are shown in Fig. 3B.

Solvent replacement with D_2O resulted in a difference spectrum with no absorbances above baseline noise levels (including the periodic baseline sine wave associated with the presence of microbubbles). As there were no positive or negative bands deviating from this baseline, it was concluded that solvent-induced changes in stratum corneum lipid conformation can be monitored accurately using solvent-replacement experiments.

75% Methanol/D_2O solvent replacement gave rise to weakly absorbing positive or negative bands (2916 and 2948.5 cm^{-1}) in the absorbance difference spectra. These subtle alterations indicate that significant alterations in lipid alkyl chain conformation or mobility were not induced with 75% methanol/D_2O solvent replacement, confirming the CH_2 band position and bandwidth data.

Solvent replacement with 75% ethanol or 2-propanol resulted in negative bands (2917.3 and 2849.7 cm^{-1}) below the baseline sine wave. The changes induced by 75% 2-propanol were greater than those induced by 75% ethanol. In some ethanol replacement experiments, only weak bands were detectable from baseline; however, 75% 2-propanol replacement exper-

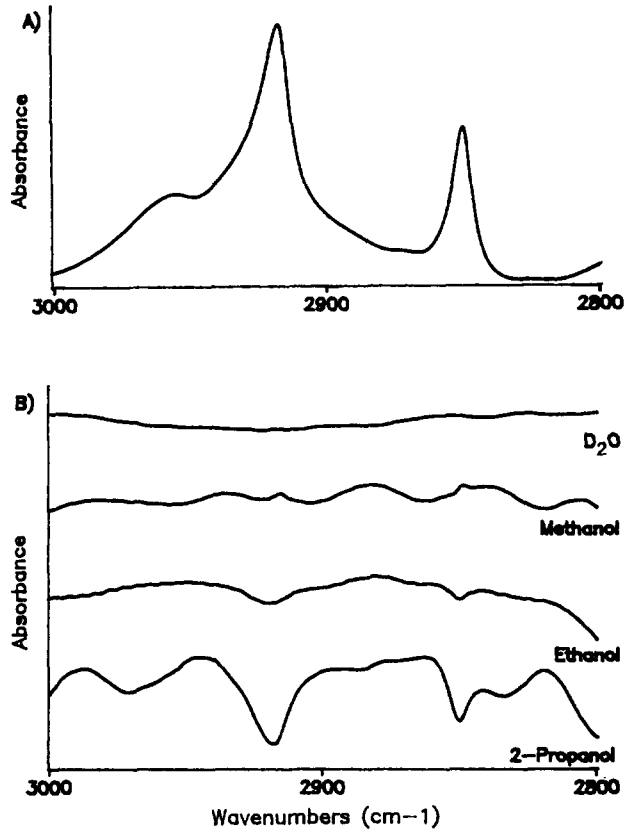

Fig. 3. CH_2 C-H stretching vibrations for 75% alcohol solvent replacement experiments: (A) stratum corneum in D_2O and (B) representative difference spectra.

iments always gave rise to definitive, negative bands. The positions of these negative bands in the absorbance difference spectra of the C-H stretching region, in conjunction with the band position and bandwidth data, suggest an overall removal of lipids from the membrane (minor changes in band position) accompanied by a redistribution of conformational populations (*trans* and *gauche*) along the alkyl chains (increased bandwidths).

Alcohol-induced alterations of the packing of stratum corneum lipid alkyl chains may be determined through deconvolution of the CH_2 C-H scissoring deformation band centered near 1468 cm^{-1}. The denseness of orthorhombic-like packing of alkyl chains in the gel phase leads to a splitting of the CH_2 scissoring band [30,36] through intermolecular coupling, which gives rise to a doublet near 1473 and 1465 cm^{-1}. When alkyl chains are packed in the less dense pseudo-hexagonal phase, greater mobility along the chains is possible; therefore, interchain interactions and the accompanying intermolecular couplings (or crystal field splitting) are reduced, which gives rise to a singlet near 1468 cm^{-1}. Hexagonal packing of liquid-crystalline alkyl chains also gives rise to a singlet near 1468 cm^{-1}. The presence of both a singlet and a doublet indicates the coexistence of multiple alkyl chain packing structures or lattices [37].

In untreated, D_2O-hydrated human stratum corneum, both a doublet and a singlet have been observed in the CH_2 scissoring deformation region [38,39], suggesting multiple lipid alkyl chain packing structures within the stratum corneum lipid matrix. No aqueous alcohol-induced changes occurred in this region after solvent replacement of D_2O with alcohol/D_2O solutions. The characteristic doublet/singlet noted on deconvolution was also observed in the presence of the alcohols (data not shown). Therefore, the distribution of lipid alkyl chains into multiple packing phases was not altered significantly in the presence of short chain alcohols (75% v/v).

3.6. Stratum corneum proteins

Infrared bands sensitive to protein conformation arise from amide carbonyl (C = O) stretching vibrational modes of the protein backbone and absorb in the Amide I region (1690–1610 cm^{-1}). The Amide I band is an envelope which is a composite of multiple bands arising from the amide carbonyl stretching vibrations. The majority of stratum corneum amide groups are within the stratum corneum proteins, although ceramides and certain amino acid side chains also absorb in this region [40,41]. Protein secondary conformations are characterized by narrow bands absorbing within specific regions of the Amide I envelope: α-helix near 1649–1656 cm^{-1}, β-sheet near 1620–1635, 1670, and 1692–97 cm^{-1}, and unordered structures near 1645–1650 cm^{-1} [42–44]. Therefore, a change in Amide I bandwidth or band shape reflects a change in distribution of protein secondary structures or conformations.

In D_2O, the Amide I band of stratum corneum is symmetric and centered near 1642 cm^{-1}, suggestive of primarily unordered conformations for the stratum corneum proteins. However, the width of the Amide I band (40–60 cm^{-1} at 70% height) implies the existence of additional protein conformations, e.g., α-helical and β-sheet conformations. In the presence of 75% (v/v) methanol, ethanol, and 2-propanol both low (1618 cm^{-1}) and high (1687 cm^{-1}) wavenumber shoulders are present within the Amide I region, indicative of β-sheet-like conformer formation (Fig. 4) [42–44].

Changes in the Amide I band position and bandwidth after solvent replacement with 75% alcohols reflect a redistribution of protein secondary structures or conformations in the presence of the alcohols. Changes in Amide I band position were greatest for 75% methanol/D_2O solvent replacement and negligible for 75% 2-propanol (Table 5). Solvent replacement with each 75% alcohol/D_2O solution resulted in a significantly increased Amide I bandwidth (Table 5),

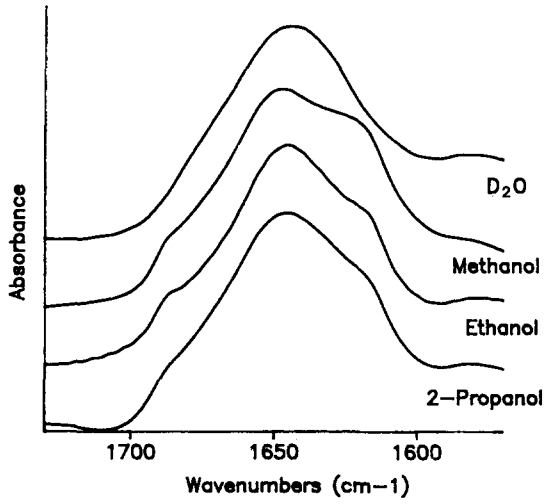

Fig. 4. Representative FTIR spectra of stratum corneum Amide I bands in the presence of D_2O and 75% alcohol/D_2O solutions.

indicating alcohol-induced changes in the distribution of stratum corneum protein conformational populations. Overall, alcohol affects on protein biophysical structures decreased with increasing alcohol lipophilicity.

The conformational alterations giving rise to the above changes in band position and bandwidth were apparent in difference spectra as well as through spectral enhancement by deconvolution and second derivatization of the Amide I band. Representative spectra of the Amide I band for stratum corneum in D_2O and 75% methanol/D_2O during a solvent replacement experiment are shown in Fig. 5. Absorbance difference spectra for representative solvent replacement experiment with D_2O or 75% alcohol/D_2O are also illustrated. The positive bands near 1688 and 1615 cm^{-1} suggest an increase in the amount of extended chains or β-sheet-like structures within the stratum corneum proteins. Difference spectra also exhibited a broad negative band near 1637 cm^{-1}, suggesting extraction of amide carbonyl-containing components and/or formation of β-sheet-like conformers at the expense of conformers absorbing near 1637 cm^{-1} (1630–1645 cm^{-1}),

Table 5
Changes in the Amide I band position (center of gravity95% ht) and bandwidth (60% ht) after solvent replacement of D_2O with 75% alcohol/D_2O solutions

Solvent	Amide I ΔCG	Amide I ΔBW	n
D_2O	−0.35 (0.23)	−0.08 (3.05)	5
75% Methanol	1.20 (0.47)	6.39 (4.31)	8
75% Ethanol	0.56 (0.48)	7.43 (2.17)	8
75% 2-Propanol	−0.52 (1.41)	6.67 (2.10)	9

Reported as mean ΔCG or ΔBW (S.D.).
$\Delta CG = CG_{Alcohol/D_2O} - CG_{D_2O}$ for each sample.
$\Delta BW = BW_{Alcohol/D_2O} - BW_{D_2O}$ for each sample.

such as extended chains or unordered conformers [42]. Formation of low and high wavenumber shoulders was greatest for 75% methanol/D_2O with shoulder formation decreasing for 75% ethanol/D_2O or 2-propanol/D_2O solutions. This suggests that methanol affects stratum corneum protein conformation to a greater extent than ethanol or 2-propanol.

Deconvolution of spectra in the presence of 75% alcohols gave bands within the Amide I envelope near 1688, 1646 and 1615 cm^{-1} (Fig. 6). Second derivative spectra showed predominant bands near 1687, 1646 and 1615 cm^{-1}. Bands near 1688 and 1615 cm^{-1} correspond to β-sheet-like conformers within the proteins [42–44] and confirm results obtained with difference spectra. It was also noted in separate experiments that β-sheet-like formation was preserved through vacuum drying of alcohol-treated stratum corneum, but was reversed upon rehydration in water or water vapor.

The extent of alcohol-induced protein changes was determined semi-quantitatively by calculating fractional changes in absorbance (Eq. (4)) near the Amide I band maximum (1644 cm^{-1}) and near the high and low wavenumber shoulder positions (1688.1 and 1618.2 cm^{-1}, respectively). The Amide I band maximum was expected to decrease as protein conformational changes and/or extraction occurred. With increasing alcohol chain length, absorbance at the maximum decreased

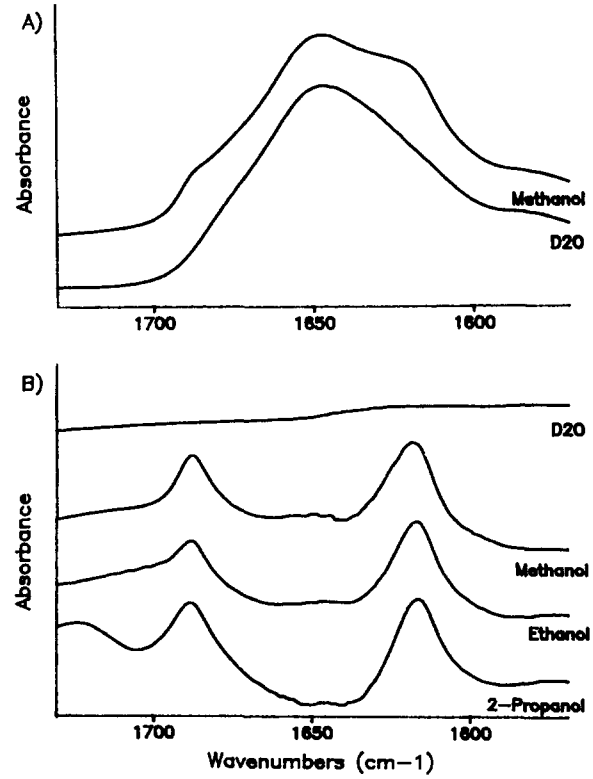

Fig. 5. Amide I band for 75% alcohol solvent replacement experiments: (A) stratum corneum in D_2O and 75% methanol/D_2O and (B) representative difference spectra.

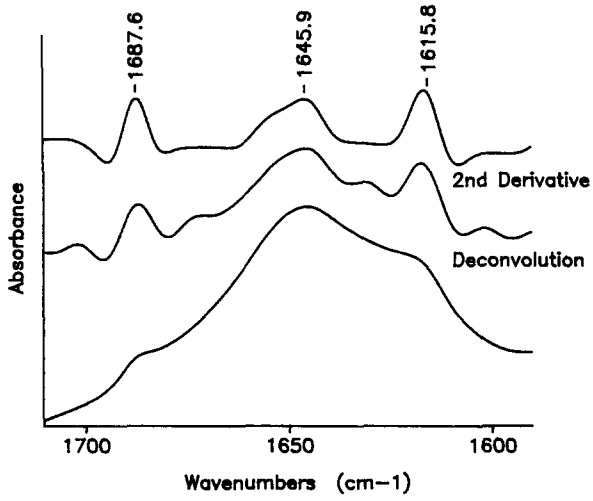

Fig. 6. FTIR absorbance spectrum, Amide I region, of stratum corneum in 75% methanol/D_2O. The same spectrum after deconvolution and derivatization (2^{nd}) is also given.

(Table 6). This progressive decrease in Amide I band maximum with increasing alcohol chain length indicates an extraction of stratum corneum components and/or formation of other conformations at the expense of extended chains or unordered conformers.

The degree of alcohol-induced perturbation of protein conformation was assessed more directly by calculating fractional changes in absorbance at the Amide I shoulders (1618.2 and 1688.1 cm^{-1}). Absorbance of these shoulders should increase with increasing formation of β-sheet-like conformations. Absorbance changes of the high and low wavenumber shoulders were greatest after 75% methanol solvent replacement and decreased with increasing alcohol chain length (Table 6). Fractional changes in absorbance were greater for the stronger-absorbing high wavenumber shoulder (1688 cm^{-1}).

The absorbance changes within the Amide I band envelope in the presence of short chain alcohols indicate that methanol effects the greatest conformational changes in stratum corneum proteins, while 2-propanol removes the greatest amount of amide carbonyl-containing components from the stratum corneum. This may be due to decreased partitioning of 2-propanol into the proteinaceous regions relative to the more polar solvent, methanol, or to an increased ability of methanol to interact with stratum corneum proteins.

Table 6
Fractional absorbance changes (Eq. (4)) in Amide I band maximum and low and high wavenumber shoulders after solvent replacement with 75% alcohol/D_2O solutions

Solvent	1688 cm^{-1}	1644 cm^{-1} (maximum)	1618 cm^{-1}	n
D_2O	0.030 (0.039)	0.010 (0.034)	0.008 (0.036)	5
Methanol	0.221 (0.053)	−0.063 (0.028)	0.081 (0.099)	8
Ethanol	0.096 (0.076)	−0.147 (0.024)	−0.025 (0.033)	8
2-Propanol	−0.053 (0.086)	−0.182 (0.054)	−0.056 (0.099)	9

Values reported as mean (S.D.).
$\Delta Abs/Abs = (Abs_{Alcohol/D_2O} - Abs_{D_2O})/Abs_{D_2O}$.

4. Discussion

It is known that short chain alcohols, in particular ethanol, increase permeability of the skin [1,7,11,45]. The data obtained in this study indicate that for similar concentrations of short chain alcohols (75% v/v), polar solute permeability coefficients increase log-linearly with increasing alcohol carbon number. However, the chain length dependence of enhanced permeation was not related to increased mannitol activity in the donor solutions, nor to an increased stratum corneum uptake of mannitol.

Insight into the diffusional characteristics of the membrane may be gained through evaluation of alcohol-induced changes in diffusional lag times. If the stratum corneum were a homogeneous membrane, lag times ($t_l = \delta^2/6D$) could be used to estimate diffusional pathlengths, δ, or diffusion coefficients, D, if either pathlength or the diffusion coefficient could be determined independently [46]. By assuming a homogeneous membrane, the measured membrane thickness is assumed to represent the diffusional pathlength. The decrease in stratum corneum thickness (Table 3) in the presence of the short chain alcohols would then imply decreased diffusional pathlength and hence decreased lag time. However, this and other studies [9,47] have shown increased lag times in the presence of the short chain alcohols, suggesting increased diffusional pathlengths, or apparent decreases in diffusion coefficients. The absence of a direct of correlation between changes in stratum corneum thickness and lag times in the presence of alcohols indicates that diffusion mechanisms are influenced directly by the presence of the aqueous alcohols.

The stratum corneum membranes were examined by FTIR in the presence of the aqueous alcohols to identify molecular level structural changes in the membrane. It is possible that in the presence of short chain alcohols, the stratum corneum lipids may experience an increased 'fluidization' as would occur with significant increases in *gauche* conformers along the lipid alkyl chains. However, no alteration of lipid alkyl chain conformation from primarily *trans* to *gauche* conformation (no shift in CH_2 stretching band positions) or gel phase packing (no change in C-H scissoring vibrations) occurred in the presence of the aqueous alcohol solutions. Thus, demonstrating that the lipids do not become more fluid in the presence of high concentrations of short chain alcohols. Rather, the increase in CH_2 stretching bandwidths, accompanied by a de-

crease in CH_2 stretching band intensity, suggested an overall extraction of and redistribution of CH_2 group conformational populations within the stratum corneum lipids. This was especially noticeable for samples in the presence of 75% 2-propanol (see increased bandwidths in Table 4). Kai et al., have also reported the extraction of hairless mouse stratum corneum lipids following 6 h treatments with ethanol, butanol, or octanol [11]. Therefore, application of alcohols may result in localized regions of greater free volume within the lipid alkyl chain regions, but does not result in an overall 'fluidization' of stratum corneum lipids.

The significant alteration of stratum corneum protein conformation by the short chain alcohols, increasing β-like conformers, may also contribute to an increased permeation of skin. However, this perturbation of membrane biophysical structure decreased with increasing alcohol chain length. Therefore, although protein conformational alterations may contribute to increased permeation to some degree, other alcohol-associated alterations in barrier function were exerted on the membrane which increased with increasing alcohol chain length. These biophysical studies have also suggested an extraction of stratum corneum lipid and protein materials which increased with increasing alcohol chain length.

5. Summary

Mannitol permeation was enhanced in the presence of three short chain alcohols: methanol, ethanol, and 2-propanol. In addition to increasing overall permeation, the alcohols increased membrane uptake of the solute, decreased stratum corneum thickness, decreased solution solubility of the solute, altered stratum corneum protein conformation, and extracted alcohol/water-soluble lipids and protein-derived components. However, the only variables which corresponded to the enhanced permeation were those suggesting extraction of stratum corneum components. Therefore, removal of stratum corneum protein and lipid components would appear to be the primary source of alcohol-enhanced permeation of polar solutes through skin. Further studies are being undertaken to determine the influence of extractable lipid and protein components on the permeation of polar solutes.

Acknowledgements

The authors gratefully acknowledge the helpful discussions with L.K. Pershing, T. Kurihara-Bergstrom and B. Berner during these studies. This research has been supported by Ciba-Geigy and NIH Grant NICHD: R01-HD-23000. CYG is a recipient of an advanced predoctoral fellowship from the PMA Foundation.

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