Biochimica et Biophysica Acta, 1112 (1992) 281–286

BBAMEM 75794

The stratum corneum lipid thermotropic phase behavior

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(Received 8 June 1992)

Key words: Stratum corneum; FTIR; Membrane structure; Gel phase packing; Lipid fluidity; Phase transition

The stratum corneum, the outermost layer of mammalian skin, is considered the least permeable skin layer to the diffusion of water and other solutes. It is generally accepted that the intercellular lipid multilayer domain is the diffusional pathway for most lipophilic solutes. Fluidization of the lipid multilayers is believed to result in the loss of barrier properties of the stratum corneum. Current investigations address the lipid thermotropic phase behavior in terms of lipid alkyl chain packing, mobility and conformational order as measured by Fourier transform infrared (FTIR) spectroscopy. A solid–solid phase transition is observed with increased alkyl chain mobility followed by a gel to liquid-crystalline phase transition near 65°C. These results further elucidate the role of lipid fluidity that may contribute to the transport properties of the stratum corneum.

Introduction

The skin is a multifunctional biological membrane. Most important of these functions is the barrier property which provides a high degree of impermeability to external foreign substances, as well as preventing water evaporation. Skin is composed of three distinct layers, the inner dermis layer, the epidermis and the outer stratum corneum layer [1]. Based on electron microscopy and biochemical techniques, Elias and coworkers [2–5] have described the stratum corneum as consisting of keratinized cell remnants embedded in an intercellular lipid matrix. The lipid matrix is comprised of numerous polar and nonpolar lipid classes [4–7].

Permeation studies of solutes through the skin have demonstrated that the stratum corneum presents the greatest barrier to percutaneous absorption [8–14]. Lipophilic solutes are believed to be transported through the lipid domain within the stratum corneum, while polar solutes diffuse through a more polar environment [8–14]. Michaels et al. [11] have suggested that the permeation of nonpolar alkanols is dependent on the ordered multilayered structure of the interstitial lipids.

The thermal dependent permeation of n-alkanols [8,12–15] and hydrocortisone [13–15] has been investigated over a wide temperature range. Knutson et al. [14] have shown that at the gel to liquid-crystalline phase transition in hairless mouse skin there is an abrupt increase in the permeability coefficients of selected solutes. It was concluded in the latter investigations that the observed thermal enhanced permeation results from fluidization of the stratum corneum lipid multilayers.

Thermally induced lipid fluidization effects have also been suggested with glucose [16–18] and water [19,20] diffusion through model membranes. Dramatic increases in the permeation of both solutes were observed at the gel to liquid-crystalline phase transition. In addition, De Kruyff et al. [21] and McElhaney et al. [22] have shown that enhanced permeation of glycerol correlates with increased fluidity of the gel phase of *Acholeplasma laidlawii* membranes. Van Deenen [23] has suggested that both the degree of packing and the mobility of the hydrocarbon chains dramatically affects transport of small molecules through lipid bilayers.

Spectroscopic techniques have been used successfully to measure the fluidity properties of biomembranes and model lipid systems. ^{31}P-NMR, ^{13}C-NMR and ^{2}H-NMR studies have demonstrated feasibility in detecting phase transitions, packing structure and conformational order of lipid polar head and alkyl chains [24–28]. The crystal lattice structures of many lipid polymorphs have been determined by X-ray diffraction [29]. X-ray diffraction has also been used to measure both the protein and lipid structures in the stratum corneum [30,31].

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Packing of model lipid bilayers has been investigated with Fourier transform infrared (FTIR) spectroscopy [28,32–40]. Casal et al. [41] and Cameron et al. [42] have demonstrated the capability of the technique to observe lipid chain mobility and conformational order prior to and during the gel to liquid-crystalline phase transition in a simple biomembrane. Alterations of the lipid polar headgroups have also been detected in phospholipid bilayers [36,38,43].

Detailed molecular level investigations of stratum corneum lipid structure are limited [13,14,44]. Current studies probe the thermotropic phase behavior of lipid multilayers within the male hairless mouse stratum corneum.

Materials and Methods

Male hairless mice (SKH-HR-1 strain, Skin Cancer Hospital, Temple University, Philadelphia, PA) between 56 and 200 days old were used as the source of stratum corneum. The procedure for separation of the stratum corneum from the epidermal-dermal layers has been previously described in detail [11–13]. Following vacuum desiccation (24 h, room temperature, 10^{-4} torr), stratum corneum abdominal sheets were stored on Teflon^{R} in a desiccator over Drierite^{R} (W.A. Hammond Drierite, Xenia, OH) until used.

Desiccated stratum corneum samples were re-hydrated by suspending the samples in a chamber with vapor in equilibrium with deuterium oxide (D_2O, Sigma, St. Louis, MO) for 24 to 25.5 h. The samples were then immersed in D_2O for 6 h. The samples were handled under a nitrogen atmosphere to prevent deuterium exchange with hydrogen. Upon completion of the hydration, the samples were sealed between two zinc selenide discs. The sealed samples were then placed in a Barnes-SpectraTech (Stamford, CT) temperature cell. Stratum corneum samples were also rehydrated in a similar manner using phosphate buffered saline (pH 7.4) and the results were similar to those hydrated in D_2O.

Transmission FTIR studies of the stratum corneum sheets were performed with a Digilab FTS 20/80 (Cambridge, MA) Fourier transform infrared spectrometer equipped with a liquid nitrogen cooled, narrow band MCT detector. Spectra (0.5 cm^{-1} resolution, 256 scans, noise level of 0.1, sensitivity of 1, zero fill factor of 2 and triangular apodization) were obtained over the mid-infrared region from 3900 to 900 cm^{-1}. Absorbance spectra were obtained at selected temperatures between 30 and 80°C over a 4-h period. Isothermal conditions (±0.25°C) were maintained with an Omega (Stamford, CT) temperature controller during data collection. The data presented are averages of three samples ± standard deviation (S.D.).

Spectral manipulations were performed with SpectraCalc (Galactic Industries, Salem, NH) and Digilab data manipulation packages. The individual spectra were baseline corrected to give a baseline at zero ($\leq \pm 0.05$ absorbance units) prior to further data manipulation. The wavenumber positions of the CH_2 asymmetric (2950 to 2870 cm^{-1}) and symmetric (2870 to 2825 cm^{-1}) stretching vibrational bands were determined by center of gravity calculations of the upper 5% of the height of the peak (95% height). The bandwidths (BW) at 70% height were also computed. The algorithms for the above computations are described by Cameron et al. [45]. The uncertainty of the band positions as determined by the center of gravity algorithm has been shown to be less than ± 0.05 cm^{-1}. The CH_2 scissoring band positions were determined after deconvolution (Digilab, halfwidth = 8, $K = 2$).

Results

A typical absorbance spectrum of male hairless mouse stratum corneum hydrated in excess D_2O at 37°C is depicted in Fig. 1. The respective lipid and protein component vibrational bands have been previously reported [13–15]. The primary lipid vibrational modes of interest are the CH_2 asymmetric (2917 cm^{-1}) and symmetric (2849 cm^{-1}) stretching modes (Fig. 1, inlay) and the CH_2 scissoring deformation modes (1472 to 1460 cm^{-1}). These vibrational bands result from the motions of the alkyl chain region of the stratum corneum lipid multilayers. The C = O stretching vibration (1740 cm^{-1}) results from the ester linkage carbonyls within the lipid polar heads.

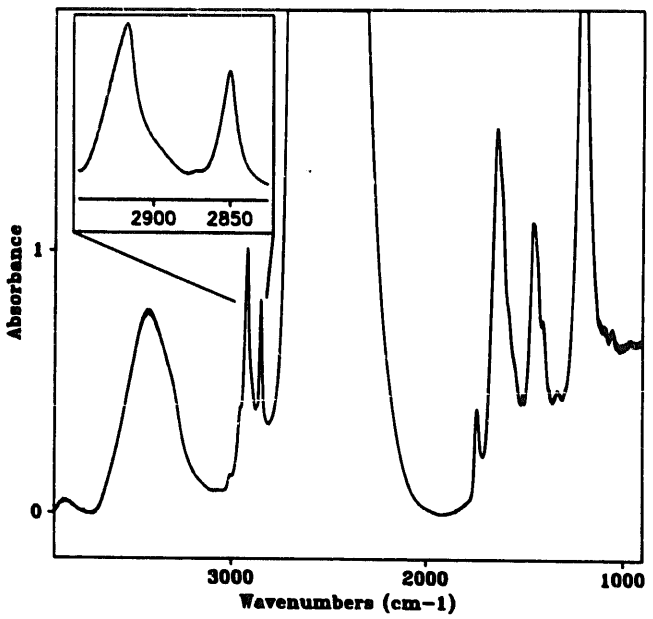

Fig. 1. Spectrum of male hairless mouse abdominal stratum corneum in excess D_2O at 37°C. Inlay: CH_2 asymmetric (2917 cm^{-1}) and symmetric (2849 cm^{-1}) stretching vibrational bands.

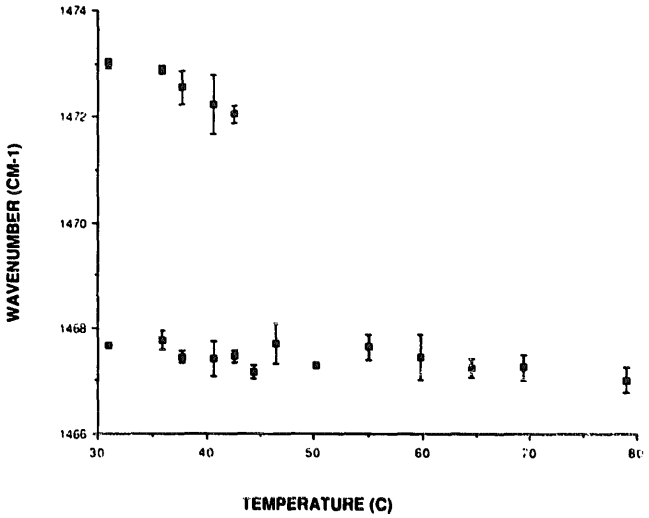

Fig. 2. Band position (cm^{-1}) of the deconvolved (halfwidth = 8, $K = 2$) CH_2 scissoring deformation vibrations (1472 cm^{-1} band of doublet and 1467 cm^{-1} singlet) as a function of temperature.

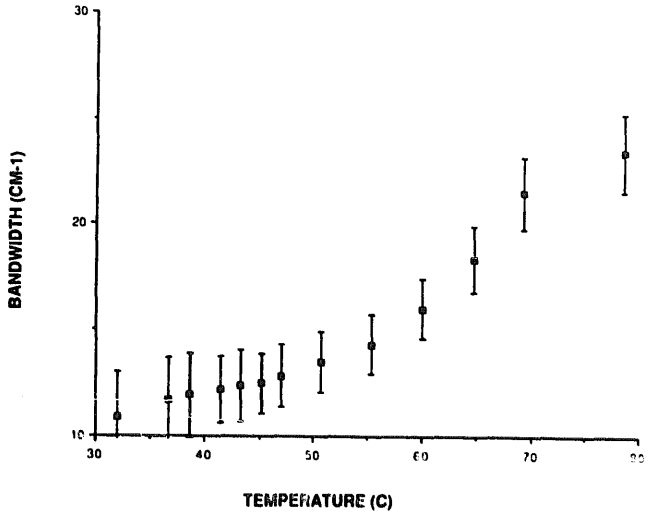

Fig. 3. CH_2 symmetric stretching bandwidth (cm^{-1}) at 70% of band height as a function of temperature.

A transition of the CH_2 scissoring doublet to a single band has been shown in model lipid systems to indicate a change in gel phase packing of the alkyl chains [28,32–39,43,48–51,53]. The presence of the CH_2 doublet (1472 and 1464 cm^{-1}) and singlet (1467 cm^{-1}) at 37°C and the singlet at 50°C indicate a solid-solid phase transition has occurred over the temperature range. The complete solid–solid phase transition gradually occurs between 31 and 43°C as illustrated in Fig. 2 which shows the wavenumber positions of the doublet's higher wavenumber shoulder and the singlet as a function of temperature. Beyond 43°C, the doublet is lost and little effect is observed. This is in agreement with the results of Almirante and co-workers [33] for model lipid bilayer systems. The presence of both the doublet and singlet below the solid-solid phase transition suggests multiple phases. are present near physiologic temperature as indicated by the X-ray diffraction studies of White et al. [31].

The bandwidth (BW) at 70% of the height of the symmetric stretching band as a function of temperature is illustrated in Fig. 3. A slight broadening of the band is observed over the temperature range of 30 to 50°C, while a dramatic broadening of the band occurs above 50°C.

The position of the CH_2 stretching vibrational bands was observed in model lipid systems to be highly sensitive to the population ratio of *trans* to *gauche* conformers [28,39,41,42,46]. Cameron et al. [42] have indicated that the absorption bands of the CH_2 asymmetric and symmetric stretching vibrations are essentially decoupled from inductive lipid polar headgroup effects. The factors that significantly affect these vibrational modes are the degree of conformational order and the freedom of motion of the alkyl chains.

Fig. 4 reveals the wavenumber position versus temperature profile of the CH_2 symmetric stretching vibrations. There is a slight increase in the wavenumber position of the band between 31 and 55°C. A more dramatic shift occurs between 55 and 80°C which has been associated with the gel to liquid-crystalline phase transition [41,42]. The gel to liquid-crystalline transition is also observed in the bandwidth measurements (Fig. 3) above 55°C. Similar behavior is observed for the CH_2 asymmetric stretching vibration over the same temperature range. The above data agree with DSC endotherms of male hairless mouse stratum corneum [13], as well as with endotherms of human stratum corneum [44,47]. The general lipid composition of the two species is similar [2].

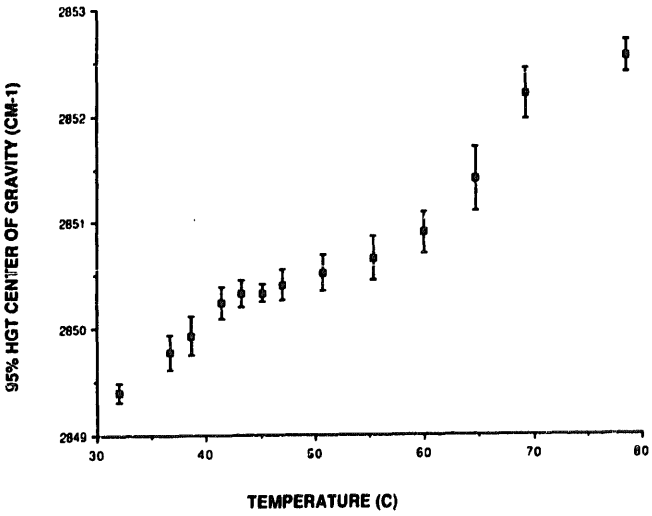

Fig. 4. CH_2 symmetric stretching band position (cm^{-1}) as determined by center of gravity at 95% of band height as a function of temperature.

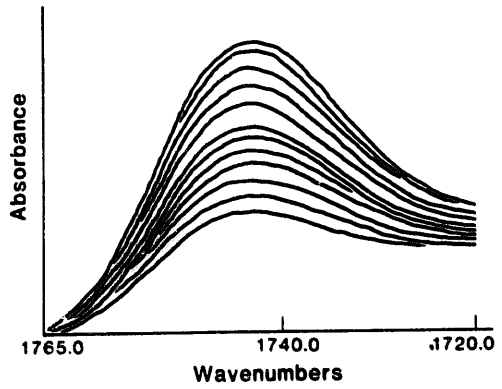

Fig. 5. Carbonyl C = O stretching vibrational band of male hairless mouse abdominal stratum corneum in excess D_2O. Temperatures of the samples in the spectra from the top are 32, 37, 39, 41, 43, 45, 47, 51, 55, 60, 65, 69 and 79°C.

The stratum corneum lipid polar heads exhibit apparent structural alterations as a function of temperature. Fig. 5 illustrates the effect of increasing temperature on the lipid carbonyl C = O stretching vibration. There is a decrease in the intensity of the band over the temperature range studied.

Discussion

Gel phase packing in simple lipid and alkane systems has been extensively studied [27,28,32–35,37–40,43,48–51]. The presence of a CH_2 scissoring doublet band has been observed in densely packed alkyl chains. This doublet results from a 'crystal field effect' [36,37,39,53] or 'factor group splitting' [33–35,43] due to interchain coupling. Studies of paraffins [48,49] suggest that the H ··· H repulsion of adjacent groups is mainly responsible for the resultant doublet formation, while minor contributions are derived from dipole–dipole interactions. Snyder [48] has shown that orthorhombic and monoclinic subcells produce a CH_2 scissoring doublet, while triclinic subcells result in a single band. The singlet has also been associated with hexagonal subcells [28,32,35–37,43].

Due to the complexity of the lipid composition in the stratum corneum, the subcell structure of the lipid alkyl chains may be a complex hybrid. The high degree of conformational order suggested by the initial position of the CH_2 symmetric stretching vibrational band (2849.4 cm^{-1}) is consistent with a densely packed hydrocarbon domain [43,52,53] possibly containing orthorhombic- or monoclinic-like subcells as indicated by the presence of the CH_2 scissoring doublet. Based on wide angle X-ray diffraction studies, White et al. [31] have suggested that stratum corneum lipid multilayers are characteristic of crystalline alkyl chains in orthorhombic-like subcells. However, a small fraction of the lipids are in the liquid-crystalline state.

Casal et al. [34] have suggested that the interchain coupling can be altered by two mechanisms: a static effect resulting in expansion of the unit subcell; and a dynamic factor related to the increased mobility of the alkyl chains. Small [54] has noted that an increase in specific volume is observed with transitions of both the solid to solid and solid to liquid-crystalline phases. For example, the transition from an orthorhombic to hexagonal packing can have approximately a 10% increase in volume. Nagle and Wilkinson [55] reported a steady increase in volume of n-alkanes upon heating prior to the solid to liquid-crystalline transition. Therefore, it is reasonable to assume that the stratum corneum lipid alkyl chains undergo a similar volume expansion with heating which is evident in the loss of interchain coupling resulting in the CH_2 scissoring singlet.

Further indication of increased volume is the enhanced freedom of torsional and vibrational motion experienced by the lipid chains as reflected in the increased bandwidth of the CH_2 stretching bands (Fig. 3) [28,39,41,42]. The increased volume is essential for the increased rotational motion of the chains about their C-C axes. With increasing temperatures approaching 60°C (Fig. 4), the broad gel to liquid-crystalline phase transition occurs giving a shift in the trans to gauche conformer population resulting in even greater chain mobility.

The high degree of structural reorganization of the alkyl chains is accompanied by a restructuring of the polar headgroup environment over the temperature range studied (Fig. 5). Dluhy et al. [43] have observed a thermal reduction in carbonyl band intensity in phospholipid bilayers. It was concluded that the reduction in intensity results from a thermally induced hydration of the carbonyl functional group. While the specific polar head alterations are not known, the waters of hydration may be displaced to the region of the bilayer near an ester linkage. These results are consistent with the permeability data of Bittman and Blau [19] where water permeability was shown to increase as a function of temperature through phospholipid bilayers.

Enhanced permeation of solutes through thermally perturbed skin is documented in the literature [12–15]. In particular, enhanced permeation of lipophilic solutes (butanol, octanol and hydrocortisone) has been observed through hairless mouse skin over the temperature range 10 to 70°C [14]. Fluidization of the intracellular lipids is hypothesized to contribute to the enhanced permeation. Van der Meer [56] has presented parameters, including self-diffusion, permeability, packing and order, which are useful estimates of fluidity in membranes. However, Seelig [26] has suggested that order and fluidity can not always be used synonymously. It is evident that the relation between

fluidity parameters is complex and not well understood.

The temperature dependence of solute permeability through phospholipid bilayers has also been demonstrated [16–18]. DPPC bilayers are considered impermeable to most solutes except for small molecules, such as glycerol, in the gel phase [20]. The inability of most molecules to permeate such a system is attributed to the densely packed alkyl chains. However, De Kruyff et al. [21] have shown the enhanced permeability of glycerol and erythritol through *Acholeplasma laidlawii* membranes over the temperature range of 0 to 20°C. DSC measurements indicate that the gel to liquid-crystal phase transition occurs at 40°C. Thermal perturbation FTIR studies of *Acholesplasma laidlawii* membranes by Casal et al. [41] demonstrate increased mobility of the alkyl chains over the same temperature range.

The current studies provide a more detailed understanding of the stratum corneum lipid thermotropic properties under similar experimental conditions where increased permeation has also been observed [14]. Enhanced permeation below 55°C may result from an increased free volume associated with the formation of a less densely packed gel phase structure rather than the melting of the lipid matrix. Assuming that the hydrocarbon region of the stratum corneum lipid multilayers can be rate limiting, then enhanced mobility of the chains through this temperature range may suggest a relationship between the expanded volume and enhanced permeation. Above the gel to liquid-crystalline transition, even greater lipid fluidity is observed with an increase in chain mobility and a decrease in conformational order. The alterations in the packing, chain mobility and conformational order support the hypothesis of decreased diffusional resistance resulting from enhanced lipid fluidity. The loosely packed gel phase is less fluid than the liquid-crystalline phase. This is qualitatively consistent with only the slightly enhanced permeation of a semi-polar compound, such as hydrocortisone, through skin prior to the gel to liquid-crystal transition, while a dramatic increase in permeability is observed above the transition [13].

In conjunction with the hydrocarbon region, the polar head plane of the lipid bilayers must be considered in the barrier properties of the stratum corneum. The carbonyl data (Fig. 5) suggest that water is entering into the initial region of the hydrocarbon domains. The increasing thermal energy may disrupt intermolecular hydrogen bonding within the region. Pascher [57] argues that interchain hydrogen bonding of polar lipids, such as ceramides, can easily account for high membrane impermeability. Studies of model solute polar functional group contributions to transport behavior through human stratum corneum suggest that if passive trans-lipid multilayer diffusion exists in the stratum corneum, the rate determining region may be near the polar headgroup region [58]. Long et al. [7] reported a significant amount of ceramide lipids with 24 and 26 ω-hydroxyacids in human stratum corneum. The presence of these lipids supports the argument for interlipid hydrogen bonding.

In summary, the current investigations have contributed to the understanding of the alkyl chain packing, mobility and conformational order in the stratum corneum lipid multilayers. The thermotropic phase behavior of the stratum corneum lipid alkyl chains can be described as a reduction in the density of packing of the chains due to a solid–solid phase transition. Enhanced mobility of the chains ensues within the enlarged volume of the system, ultimately resulting in the gel to liquid-crystalline phase transition forming a significant population of *gauche* conformers. A restructuring of the polar headgroup environment probably accompanies the alterations of the alkyl chain region of the lipid multilayers. It is evident from the data that the stratum corneum lipid thermotropic phase behavior is more complicated than a simple gel to liquid-crystalline phase transition. Thermotropic induced lipid alkyl chain packing, mobility and order, as well as the restructuring of the polar headgroup is hypothesized to be associated with the thermally enhanced permeability of lipophilic solutes through hairless mouse stratum corneum.

Acknowledgments

The support of CIBA-GEIGY and NIH grant NICHD RO1-23000 for this research is gratefully acknowledged.

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