*Skin Research and Technology 2009; **15**: 172–179*
Printed in Singapore · All rights reserved
doi: 10.1111/j.1600-0846.2008.00344.x

Skin Research and Technology

Comparative thermodynamic and spectroscopic properties of water interaction with human stratum corneum

Santosh Yadav[1], R. Randall Wickett[2], Neville G. Pinto[1], Gerald B. Kasting[2] and Stephen W. Thiel[1]

[1]*Department of Chemical & Materials Engineering, University of Cincinnati, Cincinnati, OH, USA and* [2]*College of Pharmacy, University of Cincinnati Academic Health Center, Cincinnati, OH, USA*

Background/purpose: The water content of skin has a significant impact on skin properties; sufficient hydration is necessary to keep the skin supple, flexible, and smooth. To understand more completely the water retention properties of the human skin barrier, physical macroscopic properties must be related to the structural organization of the stratum corneum (SC). Water, lipids, and natural moisturizing factor (NMF) influence the molecular structures that affect the properties of SC, including water sorption and binding enthalpy. In the research reported here, isothermal microcalorimetry was used to study the interaction of water vapor with isolated human SC in intact, delipidized, and water-washed delipidized forms to identify the influences of the principal components of SC on water sorption. The calorimetric data are interpreted in conjunction with spectroscopic results to identify the conformational changes in keratins induced by lipid and NMF removal and to assess the influence of these changes on water binding in SC.

Methods: Isothermal calorimetry was used to measure the integral heat of water vapor sorption on intact, delipidized, and water-washed delipidized human SC at 32 °C as a function of relative humidity using back and thigh skin from three donors. Calorimetric measurements were combined with water vapor sorption measurements to determine the differential thermodynamic properties of these systems. Attenuated total reflection–Fourier transform infrared spectroscopy was used to investigate effects of extraction on protein secondary structure.

Results: The magnitudes of the differential enthalpy, entropy, and free energy were greatest for intact SC and least for water-washed delipidized SC. Water sorption followed a similar trend. Delipidization led to a significantly reduced binding enthalpy at low water content; water washing the delipidized SC had only a small additional effect on binding enthalpy. Delipidization converts a fraction of keratin α-helixes to turns and random coils, while water sorption converts a fraction of keratin α-helixes to β-sheets, turns, and random coils.

Conclusions: The results of this study are consistent with a water sorption model in which keratin–keratin hydrogen bonds are replaced by keratin–water hydrogen bonds. Delipidization reduces the fraction of dry keratin that is in the α-helix conformation, suggesting that lipids hold the keratins in a conformation conducive to optimal hydration.

Key words: stratum corneum – hydration – thermodynamics – calorimetry – lipid extraction

Accepted for publication 26 November 2008

THE WATER content of skin has a significant impact on skin properties; sufficient hydration is necessary to keep the skin supple, flexible, and smooth. The stratum corneum (SC), the outermost layer of the skin, acts as a primary permeability barrier. Blank (1) showed in *in vitro* studies that human SC is flexible as long as it contains at least 10 wt% water. The degree of hydration of SC has profound effects on its mechanical and transport properties, leading to considerable interest in the cosmetics and pharmaceutical industries. The mechanical and transport properties, in turn, are greatly influenced by the microscopic structure of human SC.

SC consists of anucleate corneocytes that form non-continuous insoluble fibrous protein networks and a continuous intercellular lamellar matrix in which terminally differentiated keratinocytes are embedded (2, 3). The corneocytes are composed mainly of insoluble keratins surrounded by a cell envelope of cross-linked proteins and covalently bound lipids.

The corneocytes also contain water-soluble materials, called natural moisturizing factor (NMF), including free amino acids, organic acids, urea, and inorganic ions (4, 5). NMF accounts for 5–30% of the total dry weight of the SC (5–7). The intercellular lipids, which account for 10–25% of

the total dry weight of SC (2, 8), include equimolar amounts of ceramides, free fatty acids, and cholesterol. Although a small amount of bound water is associated with the hydrophilic polar groups of intercellular lipids such as sphingomyelin (4, 7), the corneocytes and NMF are the main components to which water binds directly (4, 6, 9–11). The water sorption capacity of SC can be reduced by treatment with organic solvents, detergents, or water; these treatments remove some of the intercellular lipids and NMF.

To understand more completely the water retention properties of the human skin barrier, macroscopic physical properties must be related to the structural organization of SC. Water, lipids, and NMF influence the molecular structures that affect the mechanical and physical properties of SC. Attenuated total reflection–Fourier transform infrared (ATR–FTIR) spectroscopy has been shown to be a powerful tool to study the molecular structure of biological tissue, and allows simultaneous investigation of proteins and lipids in SC. This technique has been used to study the extent of lipid extraction from porcine SC (8, 12) and the conformational changes and denaturation of keratins (8, 12). Rastogi and Singh (8, 12) studied the extent of extraction and conformational changes in the tissue using the Amide I band, which arises mainly from the stretching vibration of C = O. The secondary structure of proteins was estimated quantitatively using the second derivative of the Amide I band (12).

The water sorption and desorption characteristics of SC are driven by enthalpic and entropic effects that can be understood using microcalorimetry (13). Results obtained using this technique lead to an enhanced understanding of the relationship between water binding characteristics and the molecular scaffolding of SC; this improved understanding may in turn improve the design and implementation of new products and clinical strategies in both the consumer and healthcare industries.

In the research reported in this paper, isothermal microcalorimetry was used to study the interaction of water vapor with isolated human SC in intact, delipidized, and water-washed delipidized forms. This novel technique allows identification of the influences of the principal components of SC on water sorption. The calorimetric data are interpreted in conjunction with spectroscopic results to identify the conformational changes in keratins induced by lipid and NMF removal and to assess the influence of these changes on water sorption in SC.

Materials and Methods

Chemicals

All chemicals were reagent grade; detailed descriptions of all chemicals except the chemical used for the delipidization of SC are given by Yadav et al. (13). The chloroform and methanol used for delipidization of SC were purchased from Fisher Scientific (Pittsburgh, PA, USA). Distilled water was used for all studies.

Human skin and sample preparation

The intact SC samples used in this study were identified as samples Donor 1 – Back, Donor 2 – Back, and Donor 3 – Thigh in the authors' previous study, which also describes the source of the samples and sample isolation procedures (13).

Delipidized SC samples were obtained by washing the dry SC samples first with cold hexane, followed by extraction with 2 : 1 v : v chloroform : methanol at 32 °C in a shaker (New Brunswick Scientific Co. Inc., Edison, NJ, USA) (11) for 48 h. Water-washed delipidized SC was obtained by extracting the delipidized SC samples with distilled water for 1 h at 32 °C (11, 14). The amounts of materials removed by solvent extraction and water washing were determined gravimetrically. Samples were dried for 48 h at 60 torr and room temperature before and after each extraction.

Heat of sorption and water sorption

A detailed description of the experimental apparatus and methods is given by Yadav et al. (13). This study used duplicate samples (10–12 mg) of intact, delipidized, and water-extracted delipidized SC from the thigh and back of three donors.

ATR–FTIR

A Digilab Excalibur 2000 ATR–FTIR spectrometer (Digilab, Canton, MA, USA) equipped with a single reflection cell fitted with a ZnSe (refractive index $\sim$ 2.4 at 1000/cm) internal reflectance element crystal and a mercury–cadmium–telluride detector was used to obtain the spectra of SC samples. The FTIR measurements were performed using standard procedures. Sample absorbance was measured from 4000 to 600/cm.

The resolution of the FTIR was 2/cm and each spectrum was the average of 80 scans. Spectra were analyzed using the Win-IR software. The penetration depth was calculated to be approximately 2.4 μm.

To observe the conformational changes between hydrated and dry SC, samples of intact, delipidized, and water-washed delipidized SC were prepared as described above. Samples were hydrated by exposure to 80% relative humidity (RH) in the calorimeter so that the sorbed water was bound rather than free (10). The samples used for spectroscopic analysis were the same samples used in the calorimetry experiments. The samples were scanned in both up and down positions.

The region from 1450 to 1700/cm is dominated by the Amide I and Amide II protein absorption bands. The Amide I band [attributed to ν (stretching vibrations) (C = O) and ν (C – N)] was observed near 1652/cm. The Amide II band [attributed to δ (bending vibrations) (N – H), ν (C – N), and ν (C – C)] was observed near 1540/cm. The fraction of lipid extracted was estimated from the decrease in the peak areas of the asymmetric and symmetric C–H stretching bands (near 2850 and 2920/cm, respectively) (3, 12); these bands are sensitive to the alkyl chains of lipids. Structural changes in keratins were estimated using changes in the Amide I and Amide II bands. Conformational changes in the keratins were estimated by taking second derivative and Fourier self-deconvolution of the Amide I band with a bandwidth at half-height of 12/cm and an enhancement factor of 2.

The FTIR spectra were also used to calculate the relative amounts of components present and extracted from the tissue. The fraction of each component was computed by dividing the area of the component peak by the sum of areas of all component peaks, which is a measure of the concentrations of components in the tissue (8).

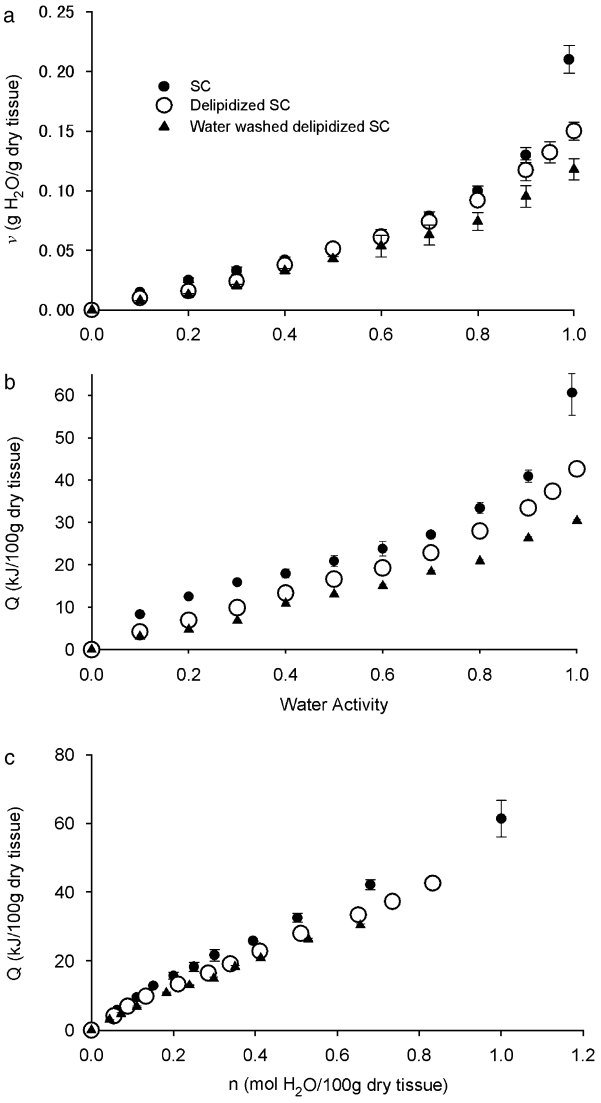

Fig. 1. (a) Water content of stratum corneum (SC) samples obtained after heat flow measurements at 32°C as functions of water activity. Integral enthalpy from heat flow measurement vs. (b) relative humidity and (c) moles of water per 100 g of dry tissue.

Results

Solvent and water extraction removed 24.6 ± 4.7 and 3.67 ± 0.58 wt% of the SC, respectively. Figure 1(a) shows the average water sorption for each SC variant as a function of the water activity in the inlet air stream. The integral heat release (Q) is shown as a function of RH (water activity) in Fig. 1(b) and as a function of the amount of water sorbed in Fig. 1(c). Water sorption and heat release were highest for intact SC and lowest for water-washed delipidized SC. Water sorption and heat release for intact SC, as well as procedures for thermodynamic analysis of the results, have been published previously (13).

Figure 2 shows the differential thermodynamic properties of water interaction with intact, delipidized, and water-washed delipidized SC as functions of the amount of water sorbed by the tissue; the results are averaged over six samples from three donors. (The units for the differential enthalpy, $\overline{\Delta H}$, and the differential free energy, $\overline{\Delta F}$, are kJ/mol water sorbed; the units for differential entropy, $\overline{\Delta S}$, are kJ/K/mol water sorbed.) The

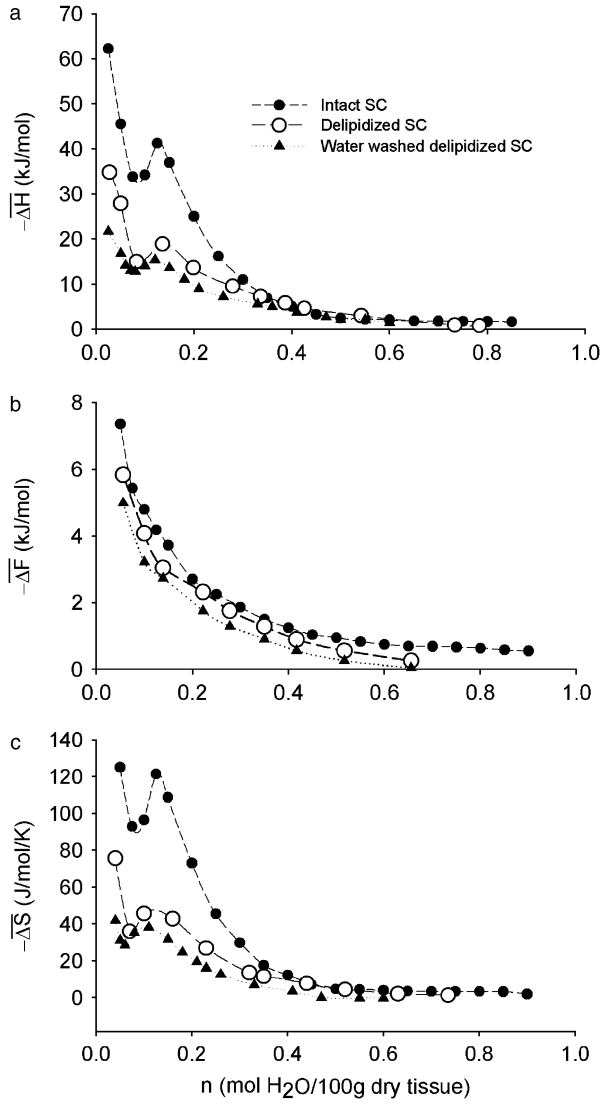

Fig. 2. Differential thermodynamic properties of stratum corneum (SC), delipidized, and water-washed delipidized SC samples as a function of moles of water sorbed; (a) differential enthalpy, (b) differential free energy change, and (c) differential entropy change.

differential properties shown in Fig. 2 have been corrected for the enthalpy and entropy of condensation, and so represent the thermodynamics of water interaction with SC. The magnitudes of the differential properties were highest for intact SC and lowest for water-washed delipidized SC. These results confirm first, that water interactions with intact SC are very strong and second, that the extractable components significantly influence the strength of the interaction of water with SC. The magnitudes of the differential free energy change, $\overline{\Delta F}$, in these tissues [Fig. 2(b)] imply that the affinities of delipidized and water-extracted delipidized SC for water are less than that of intact SC.

Representative FTIR spectra for dry and hydrated intact, delipidized, and water-washed delipidized SC are shown in Fig. 3. Figure 3(a) shows representative FTIR spectra from 1450 to 1700/cm to show the impact of delipidization and water washing on the amide bands for dry tissues; Fig. 3(b) shows the corresponding changes in the symmetric and asymmetric stretching of CH_2 bands from 2800 to 3000/cm for dry intact, delipidized, and water-extracted delipidized SC. For each peak of interest, the highest intensity was observed for intact SC, a lower intensity for delipidized SC, and the lowest intensity for water-washed delipidized SC. The same regions are shown in Fig. 3(c) and (d), respectively, after equilibration at 80% RH. The overall results obtained from FTIR (Fig. 3) follow a trend similar to that of the results obtained from thermodynamic analysis (Fig. 2).

Table 1 shows the relative changes of peak area for several functional groups of dry SC and SC hydrated at 80% RH, indicating the extent of lipid extraction, the influence of extraction on the remaining components, and the influence of hydration of each of the functional groups. Table 2 shows the results of an analysis of the Amide I band of dry SC and SC hydrated at 80% RH, illustrating the conformational changes of the keratins resulting from delipidization, water washing, and water sorption.

Discussion

Water holding mechanisms and the barrier properties of the SC have been studied over 50 years using a variety of techniques. In this study, a new approach, isothermal calorimetry, is used to quantify the thermodynamic interaction of water with different forms of SC; FTIR results are used to interpret the thermodynamic results.

Delipidization and water extraction remove substantial amounts of lipids and NMF components from SC. In this study, 25 wt% of the SC was extracted. This amount extracted is higher than that reported by Léveque et al. (11) (12 wt% extracted for 1 h at 25 °C) using chloroform : methanol. However, Yamamura and Tezuka (15) reported extracting 39.7 ± 1.7 wt% of hairless rat SC using chloroform : methanol at room temperature.

Amino acid analysis of extracts obtained in the solvent extraction of hairless rat SC (15) indicates that approximately 4 wt% of the SC is amino

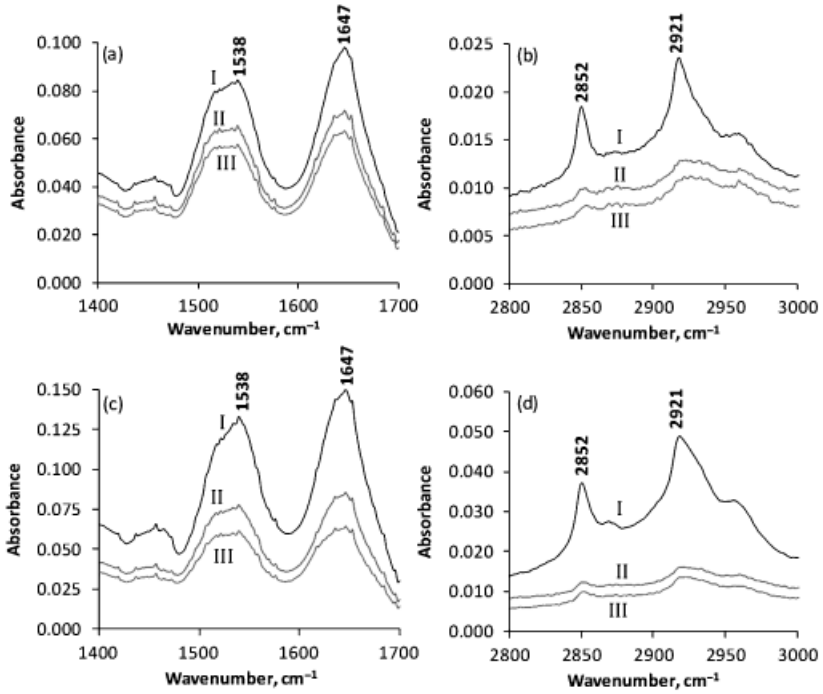

Fig. 3. Comparison of Fourier transform infrared (FTIR) spectra of dry stratum corneum (SC): intact (I), delipidized (II), and water-extracted delipidized (III) SC samples; (a) keratin-dominated region and (b) intercellular lipid-dominated region. Comparison of FTIR spectra of SC after hydration at 80% RH: intact (I), delipidized (II), and water-extracted delipidized (III) SC samples; (c) keratin-dominated region and (d) intercellular lipid-dominated region. RH, relative humidity.

TABLE 1. Influence of delipidization, water washing, and relative humidity on relative FTIR peak areas for functional groups in human SC

	Delipidized	Water-washed delipidized	Intact	Delipidized	Water-washed delipidized
	% decrease vs. dry intact SC		% increase vs. dry intact SC	% decrease vs. intact SC	
RH	0%		80%	80%	
Amide II	17.2 ± 0.6	19.1 ± 0.2	41.2 ± 1.5	31.7 ± 1.6	33.3 ± 0.5
Amide I	22.3 ± 0.4	25.5 ± 4.7	37.8 ± 2.3	33.7 ± 1.5	32.9 ± 2.5
Symmetric CH_2	76.0 ± 0.5	80.7 ± 0.1	75.5 ± 0.2	80.3 ± 0.6	81.8 ± 0.2
Asymmetric CH_2	90.0 ± 1.1	96.6 ± 0.1	45.6 ± 3.1	76.4 ± 0.8	81.5 ± 0.2

Results shown are mean ± standard deviation for three samples.
RH, relative humidity; SC, stratum corneum.

TABLE 2. Keratin conformation in human SC variants based on differentiation of the Amide I band

	Intact		Delipidized		Water-washed delipidized	
	Dry	80%	Dry	80%	Dry	80%
α-helix	55 ± 1	41 ± 2	46 ± 2	38 ± 2	45 ± 2	36 ± 4
β-sheet	31 ± 2	36 ± 2	33 ± 2	38 ± 3	33 ± 2	37 ± 2
Turns and random coils	14 ± 1	23 ± 3	21 ± 2	24 ± 1	23 ± 3	27 ± 3

Results shown are mean ± standard deviation for two samples.
SC, stratum corneum.

acids and that approximately 21 wt% of the SC is lipid, salts, and other extractable components. Chloroform : methanol extracts contain more polar lipids, such as sphingomyelin, than acetone : ether extracts. There is less bound water present in chloroform : methanol-extracted SC than acetone : ether-extracted SC; thus, polar lipids are important for water binding (15).

The effect of solvent extraction on keratins is also of interest. Norlen et al. (16) reported that chloroform : methanol-extracted SC swelled less than intact SC. Molecular analysis of the elastic properties of SC using solid-state C-NMR spectroscopy (17) revealed that removing NMF from SC caused an increase in the molecular interactions between 10 nm filaments of keratin fibers. Thus, removal of lipids and NMF leads to increased molecular interactions between keratin fibers, and so it is possible that the extraction of intercellular lipids and NMF affects the structure and polarity of keratin macrofibrils in the corneocytes.

The thermodynamics of water sorption on synthetic NMF and synthetic lipids were determined using the same apparatus and procedures used for the SC samples. Results indicate that water interactions with NMF contribute only 2% of the total integral heat evolved in water sorption by SC. Negligible amounts of water were adsorbed by the synthetic lipid. Thus, the total integral heat (after correction for latent heat) is determined primarily by water interactions with keratins.

The secondary structure of proteins is maintained in large part by hydrogen bonding between C = O and N–H groups. Lim et al. (18) presented data for water sorption in nylon consistent with a sorption mechanism in which water disrupts hydrogen bonds between the C = O and N–H groups in nylon. Similar behavior has been observed for wool (19) and cellulose (13, 20). The results presented here are consistent with the hypothesis that the changes in the differential thermodynamic properties due to extractions arise from conformational changes in keratin macrofibrils.

The intensities of the peaks near 2919/cm (asymmetric stretching of $\nu_a CH_2$) and 2850/cm (symmetric stretching of $\nu_s CH_2$) are attributed to the aliphatic chains of the lipids and some peptide side chains. The band at 2850/cm ($\nu_s CH_2$) is sensitive to the conformation of the lipid alkyl chains. The peak areas for the $\nu_a CH_2$ and $\nu_s CH_2$ bands for dry delipidized SC relative to dry intact SC suggest that 76–90% (see Table 1) of the lipids were removed by the chloroform : methanol extraction. Previous investigators found that 40-min extractions with chloroform : methanol (2 : 1) extracted 75% of intercellular lipids, where the amount extracted was determined using FTIR spectra (8, 12, 21).

Extraction of intercellular lipids and NMF also affects the amide bands [Fig. 3(a) and (b)]. These bands are sensitive to hydrogen bond interactions, including interactions with the water molecules. Intercellular lipids also contribute to the Amide I and Amide II bands; the lipid contribution, mainly from ceramide 3, is no more than 5–10% of the peak area (3). The principal reason for the decrease of the Amide I and Amide II bands in delipidized tissues is that removal of NMF and intercellular lipids increases intermolecular interaction within the keratin macrofibrils (17). As a result, the Amide I and Amide II peak areas for dry delipidized SC decrease by 22% and 17% (Table 1), respectively, as a result of delipidization.

Water washing the delipidized SC samples removed an additional 4 wt% of NMF components and resulted in an additional 4% and 7% decrease of the $\nu_s CH_2$ and $\nu_a CH_2$ peak areas and an additional 4% and 2% decrease of the Amide I and Amide II peak areas, respectively (Table 1). These decreases can be attributed to increased intermolecular interactions of keratin macrofibrils (17).

After equilibration at 80% RH, the observed intensity and peak area of each functional group increased [Fig. 3(c) and (d) and Table 1]. The increase in the intensity was highest for intact SC and lowest for water-washed delipidized SC. If water sorption results in replacement of some keratin–keratin hydrogen bonds with keratin–water hydrogen bonds, the vibrations associated with the Amide I and Amide II bands will be less constrained, resulting in an increase in peak intensity. Thus, the increases in the intensities of the Amide I and Amide II bands are consistent with the sorption model described above.

For samples equilibrated at 80% RH, the decreases in the peak areas of the Amide I and Amide II bands of delipidized SC relative to intact SC were 38% and 41% [see Table 1 and Fig. 2(c)]; the corresponding decreases of the $\nu_s CH_2$ and $\nu_a CH_2$ bands were 80% and 76% [see Table 1 and Fig. 3(d)], respectively. The water-washed delipidized SC samples equilibrated at 80% RH showed an additional decrease of 1% in Amide bands [see Table 1 and Fig. 3(c)] and a 5% decrease in the $\nu_a CH_2$ band [see delipidized SC curve in Fig. 3(d)] as compared with delipidized SC equilibrated at 80% RH. Water washing the delipidized SC resulted in no significant change

in the $\nu_s CH_2$ peak for samples hydrated at 80% RH.

The protein conformations in the samples examined by FTIR were determined through analysis of the second derivative of the Amide I bands; results of this analysis are presented in Table 2. Most of the keratin in dry intact SC is in the α-helix conformation, with smaller amounts in the β-sheet and random coil conformations. Delipidization reduces the fraction of α-helixes and increases turns and random coils; the percentage of β-sheets was not significantly changed by delipidization. Water washing the delipidized SC appears to cause no significant conformational changes.

All three SC variants showed, within the precision of the data, the same protein conformation at 80% RH. The data in Table 2 suggest that for all SC variants, water sorption is associated with the conversion of a fraction of the α-helixes to β-sheets, random coils, and turns. The maximum conversion of α-helixes occurs for intact SC, while the minimum change occurs for water-washed delipidized SC. These trends suggest that the intercellular lipids in SC hold the keratin in the corneocytes in a configuration optimized to sorb water effectively.

It appears that the conversion of α-helixes and to random coils and turns is associated with a decrease in the bound water content in delipidized SC. Imokawa et al. (6) reported that depletion of SC lipids decreased the bound water content and that the application of SC lipids to a lipid-depleted SC sheet resulted in significant recovery of bound water capacity. The results presented here support literature findings that the SC lipids hold water through formation of lamellar structures in the SC. The results presented here further suggest that a key role of the lipids is to hold the keratins in conformations that are conducive to optimal hydration.

Conclusions

The magnitudes of the differential enthalpy, $\overline{\Delta H}$, the differential free energy, $\overline{\Delta F}$, and the differential entropy, $\overline{\Delta S}$, are greatest for intact SC and least for water-washed delipidized SC. The principal thermal contribution is from the interaction of water with keratins. Spectroscopic and water sorption results are consistent with the calorimetric results. Spectroscopic results, in conjunction with the calorimetric results indicate that removing the lipid and NMF components alters the conformation of the keratins. The sorption, calorimetric, and spectroscopic results are consistent with a water sorption model in which water sorption disrupts keratin–keratin hydrogen bonds to form water–keratin bonds, which changes the confirmation of the keratins. These results suggest that lipids play a key role in maintaining the keratins in a conformation that results in optimum hydration.

References

1. Blank IH. Factors which influence the water content of the stratum corneum. J Invest Dermatol 1952; 18: 433–440.
2. Harding VR. The stratum corneum: structure and function in health and disease. Dermatol Ther 2004; 17: 6–15.
3. Garidel P. Mid-FTIR microspectroscopy of stratum corneum single cell and stratum corneum tissue. Phys Chem Chem Phys 2002; 4: 5671–5677.
4. Imokawa G, Kuno H, Kawai M. Stratum corneum lipids serve as a bound-water modulator. J Invest Dermatol 1991; 96: 845–851.
5. Rawlings AV, Harding CR. Moisturization and skin barrier function. Dermatol Ther 2004; 17: 43–48.
6. Imokawa G, Akasaki S, Kuno H et al. Functions of Lipids on Human Skin. J Dispersion Sci Technol 1989; 10: 617–641.
7. Loden M. Role of topical emollients and moisturizers in the treatment of dry skin barrier disorders. Am J Clin Dermatol 2003; 4: 771–788.
8. Rastogi SK, Singh J. Transepidermal transport enhancement of insulin by lipid extraction and iontophoresis. Pharm Res 2002; 19: 427–433.
9. Walkley K. Bound water in stratum corneum measured by differential scanning calorimetry. J Invest Dermatol 1972; 59: 225–227.
10. Kasting GB, Barai ND. Equilibrium water sorption in human stratum corneum. J Pharm Sci 2003; 92: 1624–1631.
11. Léveque JL, Escoubez M, Rasseneur L. Water–keratin interactions in human stratum corneum. Bioeng Skin 1987; 3: 227–242.
12. Rastogi SK, Singh J. Passive and iontophoresis transport enhancement of insulin through porcine epidermis by depilatories: permeability and Fourier transform infrared spectroscopy studies. AAPS Pharm Sci Tech 2003; 4: 1–9.
13. Yadav S, Pinto NG, Kasting GB. Thermodynamics of water interaction with human stratum corneum. I. Measurement by isothermal flow calorimetry. J Pharm Sci 2007; 96: 1585–1597.
14. Tanojo H, Bouwstra J, Junginer HE et al. Subzero thermal analysis of human stratum corneum. Pharm Res 1994; 11: 1610–1616.
15. Yamamura T, Tezuka T. The water-holding capacity of the stratum corneum measured by 1H-NMR. J Invest Dermatol 1989; 93: 160–164.
16. Norlen L, Emilson A, Forslind B. Stratum corneum swelling, biophysical and computer assisted quantitative assessments. Arch Dermatol Res 1997; 289: 506–513.

17. Jokura Y, Ishikawa S, Tokuda H et al. Molecular analysis of elastic properties of the stratum corneum by solid-sate C-nuclear magnetic resonance spectroscopy. J Invest Dermatol 1995; 104: 806–812.
18. Lim LT, Britt IJ, Tung MA. Sorption and transport of water vapor in nylon 6,6 film. J Appl Polym Sci 1999; 71: 197–206.
19. Morrison JL, Hanlan JF. Swelling of fibrous proteins. Nature 1957; 179: 528–529.
20. Hollenbeck RG, Peck GE, Kildsig DO. Application of immersional calorimetry to investigation of solid–liquid interactions: microcrystalline cellulose–water system. J Pharm Sci 1978; 67: 1599–1606.
21. Singh J, Rastogi SK. Lipid extraction and transport of hydrophilic solutes through porcine epidermis. Int J Pharm 2001; 225: 75–82.

Address:
Stephen W. Thiel
Department of Chemical and Materials Engineering
University of Cincinnati
Cincinnati, OH 45221-0012
USA
Tel: +1 513 556 4130
Fax: +1 513 556 3474
e-mail: stephen.thiel@uc.edu