Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3871–3873, May 1990
Biophysics

Lipid biophysics of water loss through the skin

(stratum corneum/permeability/lipid disorder/infrared spectroscopy)

RUSSELL O. POTTS* AND MICHAEL L. FRANCOEUR

Pfizer Central Research, Groton, CT 06340

Communicated by Donald M. Crothers, March 14, 1990 (received for review January 16, 1990)

ABSTRACT **The regulation of water loss through the skin is a poorly understood but crucial process in maintaining terrestrial life-forms. In mammalian skin, the outermost layer, called the stratum corneum (SC), is rate-limiting to water loss. We have evaluated temperature-dependent changes in water vapor permeability and infrared spectra of porcine SC. In particular, we have analyzed the infrared absorption peaks due to the extracellular lipids of the SC. These results show a remarkable correlation between water permeability and the frequency of the C—H stretching vibrations over a broad range of temperature. Since the spectral changes reflect an increased number of alkyl gauche conformers, these results suggest that water permeability is dependent upon the hydrocarbon-chain disorder of SC lipids.**

While all life-forms depend on membrane-like structures for their existence, nowhere is that more dramatically demonstrated than in the skin. This is particularly true in higher mammals, where this complex membrane has evolved into a multifunctional organ. In terrestrial animals, one of the skin's most vital functions is to regulate the amount of water lost to the environment. This crucial regulatory function is achieved by a remarkable structure known as the stratum corneum (SC), the thin, outermost layer of the skin. The SC is composed of alternating layers of flat, protein-rich cells surrounded by an extracellular lipid matrix (1, 2) in an array similar to "bricks and mortar" (1). The extracellular lipids form an extended multilamellar domain and provide the only continuous phase from top to bottom of this tissue. Biophysical evidence from our laboratory suggests that SC lipid domains are the primary barrier to water loss (3) and to penetration of small compounds into the skin (4). In addition, removal of lipids from the SC by solvent extraction leads to a 1000-fold increase in water permeability (5). Thus, the role of SC lipids in regulating water loss is well established.

The mechanism by which water passes through the SC, or any other lamellar lipid phase, is not well characterized. A number of investigators have suggested that water permeates through lipid lamellae via free-volume voids created due to random fluctuations in alkyl chain packing (6, 7). In this investigation, we have compared temperature-induced changes in SC water vapor permeability with lipid conformational changes determined by Fourier-transform infrared (IR) spectroscopy. The findings show a strong correlation between water permeability and SC lipid alkyl-chain disorder and, thus, support the free-volume hypotheses.

MATERIALS AND METHODS

Permeability Measurements. Water vapor transport was measured by adapting the technique described by Blank *et al.* (8). These measurements were made by placing a sheet of isolated SC between two halves of a vapor permeation cell. Two milliliters of a saturated aqueous solution of NaCl was introduced into both sides of the permeation cell, and the entire apparatus was immersed in a water bath at the appropriate temperature. Saturated aqueous NaCl was chosen because it maintains 75% relative humidity from 10°C to 90°C. After several days equilibration, tritiated water [$^1H^3HO$ (HTO), 1 mCi/ml, New England Nuclear; 1 mCi = 37 MBq] was introduced into the donor chamber through a septum. Aliquots were removed from the receiver chamber with increasing time and analyzed by liquid scintillation counting. From the slope of the cumulative radioactivity-vs.-time data, the rate of accumulation was calculated (dpm/hr). This value, when divided by the cross-sectional area available for permeation and by the specific activity of HTO, yielded the steady-state flux [mg/(cm^2·hr)]. The steady-state flux, when divided by the concentration of permeant on the donor side, yielded the permeability constant (cm/hr).

The use of vapor permeability techniques has several advantages. First, by using saturated NaCl solutions, the water activity (relative humidity) was maintained constant throughout the temperature range studied. Second, unstirred-layer effects, which can be appreciable in condensed phases, were negligible in these experiments. Thus, temperature-induced changes in permeability do not reflect changes in permeant activity or the resistance of an unstirred layer. Finally, the SC acts *in vivo* as a barrier to water vapor permeation and, thus, the results are physiologically relevant.

IR Spectra. Spectra of porcine SC were obtained as described (9). In brief, samples were equilibrated for at least 12 hr at room temperature and 75% relative humidity. The sample was then sealed between two IR-transparent windows and mounted in a temperature-controlled holder (Spectra Tech, Stamford, CT). The temperature was manually regulated with a constant-temperature bath (Lauda model RC-6, Brinkmann) and monitored with a thermocouple in direct contact with the sample through a small hole in the window. Spectra were obtained at about 10°C intervals from 22°C to 90°C. The data were obtained using a Fourier-transform IR spectrometer (model 730, Nicolet) with an instrumental resolution of 0.5 cm^{-1}. The frequency of maximal absorbance was determined to within 0.1 cm^{-1} by using software provided by Nicolet (10). At least three scans of 64 interferograms were co-added for each spectrum.

RESULTS AND DISCUSSION

Temperature-dependent changes in SC water vapor permeability (P) and the CH_2 symmetric C—H stretching frequency (ν_{CH2}) are presented in Table 1. These data are similar to those published previously (3), with several notable exceptions. The precision of the permeability data has been increased by the evaluation of more samples. Similarly, the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SC, stratum corneum; P, water vapor permeability; ν_{CH2}, C—H symmetric stretching frequency.

*To whom reprint requests should be addressed.

Table 1. Temperature dependence of porcine SC water permeability and C—H symmetric stretching frequency

Temperature, °C	P × 10^3, cm/hr	ν_{CH2}, cm^{-1}
22	0.44 ± 0.03 (33)	2849.8 ± 0.1 (4)
30	1.08 ± 0.09 (16)	2850.1 ± 0.1 (5)
40	1.94 ± 0.17 (14)	2850.2 ± 0.1 (5)
50	3.92 ± 0.36 (13)	2850.3 ± 0.1 (5)
60	7.55 ± 0.45 (10)	2850.7 ± 0.1 (5)
70	21.1 ± 3.0 (12)	2851.6 ± 0.1 (5)
80	27.8 ± 2.0 (9)	2852.7 ± 0.4 (4)
90	32.5 ± 5.3 (12)	2853.3 ± 0.3 (4)

Values are presented as mean ± SEM. For each value, the number of replicate samples is given in parentheses.

precision of the IR data was increased in this study through the use of replicate samples. Finally, the Fourier-transform IR instrument used in this study has much greater spectral resolution than that used previously (0.5 vs 2.7 cm^{-1}).

The permeability results (Fig. 1 *Upper*) show that P increased with increasing temperature, with an abrupt rise between 50°C and 80°C. The permeability value obtained at 30°C is in excellent agreement with water permeation values obtained previously for human (5, 8, 11) and porcine (12) SC. Similarly, the spectral results (Fig. 1 *Lower*) show a continuous increase in ν_{CH2} as the temperature is increased, with an abrupt change from 50°C to 80°C. The dependence of ν_{CH2} on temperature illustrated here is identical to IR results obtained for a number of biomembranes and is associated with the progressive disordering of lipid alkyl chains (13, 14). Furthermore, calorimetric evaluation of porcine SC has revealed two broad lipid thermal transitions occurring over the same temperature range (3, 4, 9, 15), in agreement with the IR results presented here.

The temperature-dependent changes in P and ν_{CH2} for porcine SC are shown in Table 1 and Fig. 2. A linear regression analysis of ν_{CH2} vs. P at each temperature shows that these variables are highly correlated ($r > 0.999$; $P_{corr} > 0.9999$). In addition, no experimental value differs significantly ($P > 0.95$; t test) from the predicted value, suggesting that these variables are functionally related. The results of previous studies with porcine (3), human (9), and murine (15) SC showed that the temperature-induced shift in ν_{CH2} was primarily due to changes in extracellular lipids. Furthermore, it has been demonstrated for a number of lipid systems that the magnitude of the increase in ν_{CH2} correlates with the number of gauche conformers (14). Hence, the correlation between P and ν_{CH2} shown here suggests that the temperature-induced increase in water permeation through the SC is dependent primarily on the formation of gauche conformers in the lipid alkyl chains. A similar correlation between temperature-induced changes in ν_{CH2} and membrane leakage has been reported for pollen particles and attributed to

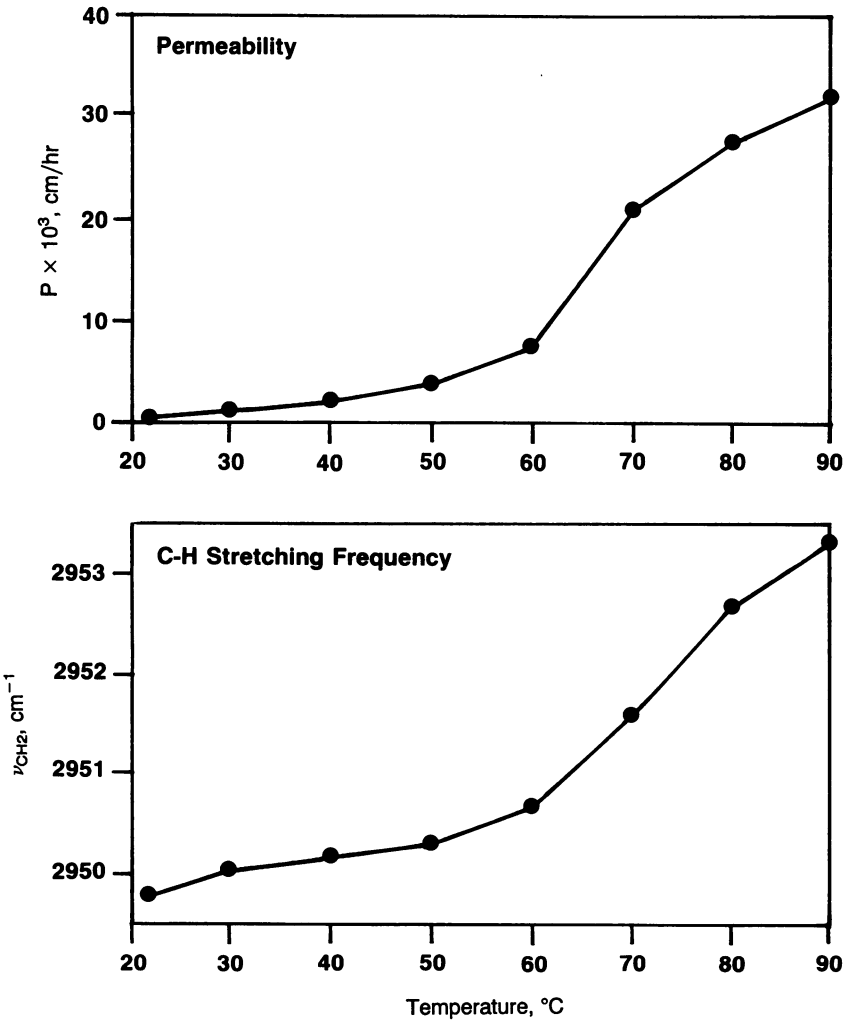

FIG. 1. (*Upper*) Water vapor permeability (P) of porcine SC as a function of temperature. All experiments were performed at 75% relative humidity. (*Lower*) The C—H symmetric stretching frequency (ν_{CH2}) of porcine SC as a function of temperature for samples equilibrated at 75% relative humidity. Samples were treated identically as those used for permeability measurements.

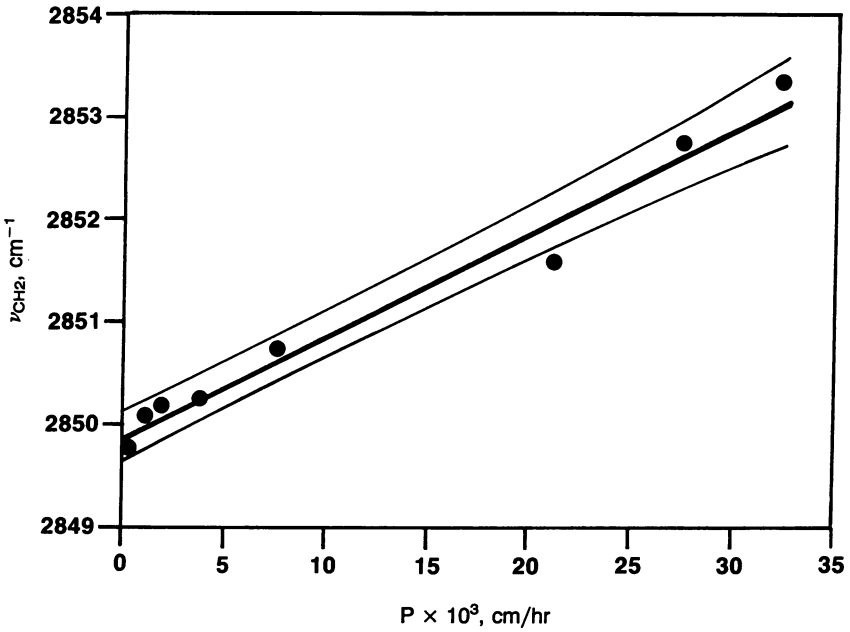

FIG. 2. Data of Fig. 1 replotted as ν_{CH2} vs. P for porcine SC. The best-fit straight line and 95% confidence interval generated from the linear regression analysis are also shown.

changes in permeability that are dependent on lipid alkyl-chain conformation (16).

While it is possible that the correlation between P and ν_{CH2} reflects a fortuitous relationship between two activated but unrelated processes, several lines of evidence argue against this. First, results from our laboratory (3) and from Elias (17) have demonstrated the crucial role of SC lipids in limiting water permeability through the skin. More importantly, the data in Fig. 2 show that P and ν_{CH2} are correlated at all measured temperatures, including those above and below the midpoint of the SC lipid phase transition (T_m), which occurs near 70°C in porcine SC (3, 4, 9, 15). This correlation requires that both processes share a common T_m and display a similar temperature dependence on either side of T_m, an unlikely chance event. Thus, the correlation most likely reflects a functional relationship between SC water permeability and lipid alkyl-chain conformation.

In addition to providing information on the biophysics of water transport through the skin, the results presented here shed light on the mechanism of water permeation through lipid bilayers in general. Two related mechanisms have been postulated for the permeation of water across biological membranes (6, 7). They involve the formation of free-volume defects in the membrane. Lieb and Stein (6) proposed that permeant molecules were transported by randomly "jumping" from donor to acceptor "holes." Alternatively, Trauble (7) proposed that water permeation through lipid bilayers involves the propagation of water-carrying "kinks" along the alkyl chain. Furthermore, he postulated that the "kinks" occurred due to the random (e.g., thermal) occurrence of gauche conformers in an otherwise primarily trans lipid alkyl chain. The data in Fig. 1 suggest that water permeation through the SC is highly correlated with the number of gauche conformers (as measured by ν_{CH2}). While not distinguishing between the "jump" or "kink" propagation hypotheses, these results nevertheless provide strong evidence that water permeation through a lipid membrane occurs via formation of gauche-conformer, free-volume "holes." To our knowledge, this is the first experimental evidence directly linking water permeation to the number of gauche conformers in a lipid membrane system.

In conclusion, water transport through the SC is highly correlated with spectral measures of lipid alkyl-chain disorder. These results, in addition to demonstrating the crucial importance of SC lipids to the skin's barrier to water loss, provide insight into the underlying biophysics of water transport and suggest that permeation is related to the number of gauche conformers formed in the lipid alkyl chain.

We thank Drs. Richard Guy, Bill Curatolo, Jim McKie, Vivien Mak, Eric Wakshull, and George Milne for many valuable suggestions.

1. Williams, M. L. & Elias, P. M. (1987) *CRC Crit. Rev.* **3**, 95–122.
2. Wertz, P. W., Swartzendruber, D. C., Abraham, W., Madison, K. C. & Downing, D. W. (1987) *Arch. Dermatol. Res.* **123**, 1381–1384.
3. Golden, G. M., Guzek, D. B., Kennedy, A. H., McKie, J. E. & Potts, R. O. (1987) *Biochemistry* **26**, 2382–2388.
4. Golden, G. M., McKie, J. E. & Potts, R. O. (1987) *J. Pharm. Sci.* **76**, 25–28.
5. Blank, I. H. (1952) *J. Invest. Dermatol.* **18**, 433–440.
6. Lieb, W. R. & Stein, W. D. (1969) *Nature (London)* **224**, 240–243.
7. Trauble, H. (1971) *J. Membr. Biol.* **4**, 193–208.
8. Blank, I. H., Moloney, J., Emslie, A. G., Simon, I. & Apt, C. J. (1984) *J. Invest. Dermatol.* **82**, 188–194.
9. Golden, G. M., Guzek, D. B., Harris, R. R., McKie, J. E. & Potts, R. O. (1986) *J. Invest. Dermatol.* **86**, 255–259.
10. Jones, R. N. & Seshadri, K. S. (1962) *Can. J. Chem.* **40**, 334–340.
11. Scheuplein, R. J. & Blank, I. H. (1971) *Physiol. Rev.* **57**, 702–747.
12. Galey, W. R., Lonsdale, H. K. & Nacht, S. (1976) *J. Invest. Dermatol.* **67**, 713–717.
13. Cameron, D. G., Martin, A. & Mantsch, H. H. (1983) *Science* **219**, 180–182.
14. Cameron, D. G., Martin, A., Moffatt, D. J. & Mantsch, H. H. (1985) *Biochemistry* **24**, 4355–4359.
15. Knutson, K., Potts, R. O., Guzek, D. B., Golden, G. M., McKie, J. E., Lambert, W. J. & Higuchi, W. I. (1985) *J. Controlled Release* **2**, 67–87.
16. Crowe, J. H., Hoekstra, F. A. & Crowe, L. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 520–523.
17. Elias, P. M. (1988) *Drug Dev. Res.* **13**, 97–105.