Does Hydration Affect Intercellular Lipid Organization in the Stratum Corneum?

Vivien H. W. Mak,[1,3] Russell O. Potts,[2] and Richard H. Guy[1]

Received September 19, 1990; accepted February 19, 1991
KEY WORDS: stratum corneum; skin hydration; infrared spectroscopy.

INTRODUCTION

It has now been demonstrated that the skin provides a viable route of delivery for potent, systemically active drugs. Further, topical application of dermatological drugs is the logical choice for the treatment of skin diseases. In both situations, the medication is often administered under occlusive conditions, in which the application site is covered, normal transepidermal water loss is impeded, and skin (and, in particular, stratum corneum) hydration is increased. Generally, hydration of the stratum corneum (SC), the principal barrier to transdermal drug delivery, is believed to enhance the rate of percutaneous absorption (1). However, the mechanism by which this effect is induced remains unclear. The SC consists of terminally differentiated keratinocytes embedded in a lamellar intercellular lipid matrix (2). Recent research has suggested that the continuous lipid domain constitutes an important, tortuous pathway for molecular transport through the SC (3). Significantly, it has been shown that water permeability across the SC is highly dependent upon the hydrocarbon-chain disorder of the SC lipids (4). It is logical, therefore, to ask the question, "Does hydration affect intercellular lipid organization in the SC?" The answer should permit one to hypothesize a causative link between hydration and enhanced SC permeability. In this note, we address this issue using the technique of infrared spectroscopy (5).

MATERIALS AND METHODS

Human and porcine stratum corneum (HSC and PSC, respectively) were obtained and prepared as described previously (6). Following isolation from the epidermis, SC was dried *in vacuo* overnight. The dehydrated SC was suspended in 99.5% D_2O (Cambridge Isotope Laboratories, Cambridge, MA) for approximately 8 hr and was then dehydrated again as before. After this second dehydration, a section of the deuterium-exchanged SC was subjected to infrared (IR) spectroscopy (Analect FX-6200 Fourier transform infrared spectrometer, Irvine, CA). The sample was sealed between two IR-transparent, ZnS windows, and the ensemble was mounted in the path of the IR beam. The sequence of dehydration, hydration with D_2O, and dehydration again was designed (a) to remove as much H_2O from the SC as possible, (b) to exchange any tightly associated H_2O with D_2O, and then (c) to dehydrate the membrane in preparation for the controlled hydration procedure below.

Further pieces of SC were placed into either a D_2O-saturated or a H_2O-saturated hydration chamber (containing saturated potassium sulfate solution which maintained the relative humidity at 95%). Every 30 min thereafter, a piece of SC was removed, weighed (to measure the percentage weight gain due to water uptake), and subjected to IR spectroscopy. The effect of hydration on SC lipid organization was evaluated by the frequency shift in the absorbance due to the C–H asymmetric stretching vibration, which occurs at about 2920 cm^{-1}. It has been shown that this IR spectral feature originates predominantly from the SC intercellular lipid hydrocarbon chains (6).

All spectra were recorded at ambient temperature (20–25°C) and represented the average of 64 scans collected over a 2-min period. The instrumental resolution (2.7 cm^{-1} was improved to 0.1 cm^{-1} using a center-of-gravity algorithm (7).

RESULTS AND DISCUSSION

It should first be noted that the procedure for preparing dehydrated SC did not remove the most tightly bound H_2O molecules. It was still possible to observe a residual O–H stretching vibration signal in the spectrum of the dehydrated membrane (data not shown).

Representative spectra of HSC hydrated for a period of 2 hr in H_2O and in D_2O are shown in Figs. 1A and B, respectively. Hydration with H_2O leads to a substantial augmentation of the broad O–H stretching absorbance centered at about 3200 cm^{-1}. On the other hand, uptake of D_2O does not significantly alter the spectrum in this region, but it does cause the appearance, as expected, of a new, large absorbance (O–D stretching) at about 2500 cm^{-1}.

For other biomembranes, it has been previously reported that an increased level of hydration causes the lipid hydrocarbon chain-associated C–H asymmetric stretching absorbance maximum to shift to a higher wavenumber (8). The interpretation of this observation is that the lipid-chain disorder has been increased; that is, there is an increased number of gauche conformers in the lipid alkyl chains, reducing the efficiency of packing and causing the energy needed to vibrate the C–H bonds to increase (5). For SC progressively hydrated in H_2O, it appeared that this pattern of behavior was reproduced (Fig. 2). However, reexamination of Fig. 1A shows that the large increase in the O–H absorbance can "bias" the peak maxima of the C–H asymmetric and symmetric stretching absorbances (which occur at around 2920 and 2850 cm^{-1}, respectively). In other words, the greatly enhanced O–H band causes the C–H absorbances to be located on the shoulder of the broad peak centered at 3200 cm^{-1}. It is possible, therefore, that this

[1] Departments of Pharmacy and Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, California 94143.
[2] Cygnus Therapeutic Systems, Redwood City, California 94063.
[3] To whom correspondence should be addressed at Dermal Therapeutics Group, Central Research Division, Pfizer Inc., Groton, Connecticut 06340.

0724-8741/91/0800-1064$06.50/0

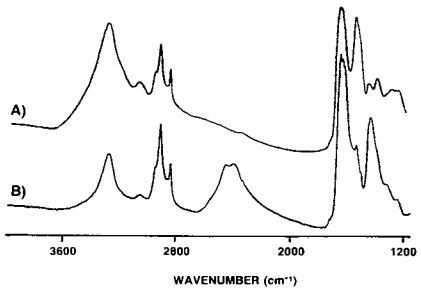

Fig. 1. Representative IR spectra of HSC hydrated for 2 hr at 95% relative humidity in (A) H_2O and (B) D_2O.

effect shifts the apparent maxima of the C–H absorbances to higher wavenumbers. The experiments using D_2O were obviously designed to circumvent this problem. As stated above, the O–D absorbance occurs at much lower energy and does not interfere with the location of the C–H absorbances (see Fig. 1B). The D_2O data in Fig. 2 confirm the suggestion that hydration with H_2O can artifactually influence the apparent position of the SC lipid C–H absorbances. When hydrated with D_2O, these absorbances show no significant shift to higher (or, for that matter, lower) wavenumber. We deduce, therefore, that SC hydration does not lead to an overall increase in intercellular lipid disorder.

Although this observation provides an exception to much of the biomembrane literature, the IR findings have very recently been substantiated by X-ray diffraction experiments. Using small-angle X-ray scattering, Bouwstra *et al.* (9) have shown that the interlamellar repeat (or so-called *d*-spacing) of HSC lipids is not altered by hydration over the range 0 to 40% (w/w). They concluded that increasing the water content of the SC causes "no swelling of the lipid bilayers." Similar results have also been obtained by Hou *et al.* using murine SC (10); these authors report the same *d*-spacing both for dry samples and for samples exposed to excess water. Further, circumstantial support comes from differential scanning calorimetry (DSC). While SC hydration causes the phase transition temperatures (T_m) of the intercellular lipids to decrease by 5–8°C (6), these effects are relatively small compared to changes of up to 80°C which have been measured for phospholipid systems (11). An explanation for this small to negligible effect of hydration on SC lipids may be the low solubility of water in the lamellae. Direct measurements of water uptake by SC lipids, and analysis of the water-dependent freezing-point depression of SC lipid transitions, suggest that SC lipids are maximally hydrated at less than 1 water molecule per lipid (3). In contrast, lamellar phospholipid systems can take up more than 10 water molecules per lipid, even in the solid phase (11). Given that the head-groups of SC lipids are significantly less polar than those of phospholipids (12), it is reasonable to expect that SC lipids have less affinity for water.

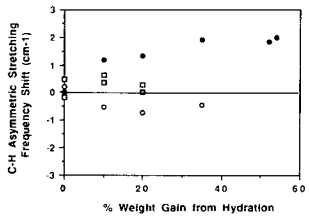

Fig. 2. Effect of hydration on the maximum frequency of the C–H asymmetric stretching absorbance from the intercellular lipids of (a) PSC hydrated at 95% relative humidity (RH) in H_2O (filled circles; each data point is the average of two separate measurements), (b) PSC hydrated at 95% RH in D_2O (open circles), and (c) HSC hydrated at 95% RH in D_2O (open squares; each data point is the average of two separate measurements). Hydration of the SC in H_2O causes the C–H asymmetric stretching absorbance frequency to shift to a significantly higher wavenumber. Hydration with D_2O, on the other hand, leads to no significant change (higher or lower) in wavenumber at any level of weight gain.

The question remains, "How does hydration lead to enhanced molecular transport across the SC?" The results presented in this paper strongly imply that increased lipid alkyl-chain disorder is *not* the causative mechanism. Alternative hypotheses, which should now be explored, include (a) the possibility that hydration induces lipid phase separation (thereby creating interfacial defects and reducing diffusional resistance), as has recently been suggested for oleic acid (12), and (b) the potential action of water on other structural domains of the SC, such as the intracellular keratin.

ACKNOWLEDGMENTS

This work was supported by Grant HD-23010 from the National Institutes of Health. We thank Dr. Michael L. Francoeur for his comments and advice and Dr. S. Y. Hou *et al.* for allowing us to quote their data prior to publication.

REFERENCES

1. B. W. Barry. *Dermatological Formulations: Percutaneous Absorption,* Marcel Dekker, New York, 1983.
2. P. M. Elias. Plastic wrap revisited. *Arch. Dermatol.* **123**:1405–1406 (1987).
3. R. O. Potts and M. L. Francoeur. The influence of stratum corneum morphology on water permeability. *J. Invest. Dermatol.* (in press).
4. R. O. Potts and M. L. Francoeur. The lipid biophysics of water loss through the skin. *Proc. Natl. Acad. Sci. USA* **87**:3871–3873 (1990).
5. H. L. Casal and H. H. Mantsch. Polymorphic phase behavior of phospholipid membranes studied by infrared spectroscopy. *Biochim. Biophys. Acta* 779:381–401 (1984).
6. G. M. Golden, D. B. Guzek, R. R. Harris, J. E. McKie, and R. O. Potts. Lipid thermotropic transitions in human stratum corneum. *J. Invest. Dermatol.* **86**:255–259 (1986).
7. D. G. Cameron, J. K. Kauppinen, D. J. Moffatt, and H. H. Mantsch. Precision in condensed phase vibrational spectroscopy. *Appl. Spectrosc.* **36**:245–250 (1982).
8. R. L. Amey and D. Chapman (1983). IR spectroscopic studies of model and natural biomembranes. In D. Chapman (ed.), *Biomembrane Structure and Function,* Macmillan, London, pp. 199–256.
9. J. A. Bouwstra, M. A. de Vries, W. Bras, J. Brussee, and G. S. Gooris. The influence of C_n azones on the structure and thermal behavior of human stratum corneum. *Proc. Int. Symp. Control. Rel. Bioact. Mater.* 17:33–34 (1990).
10. S. Y. Hou, A. K. Mitra, S. H. White, G. K. Menon, R. Ghadially, and P. M. Elias. Membrane structures in normal and essential fatty acid deficient stratum corneum: characterization by ruthenium tetroxide staining and x-ray diffraction. *J. Invest. Dermatol.* (in press).
11. D. M. Small. *The Physical Chemistry of Lipids,* Plenum Press, New York, 1986.
12. M. L. Francoeur, G. M. Golden, and R. O. Potts. Oleic acid: Its effects on stratum corneum in relation to (trans)dermal drug delivery. *Pharm. Res.* 7:621–627 (1990).