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Report

Application of Solid-State Nuclear Magnetic Resonance (NMR) to the Study of Skin Hydration

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The solid-state nuclear magnetic resonance (NMR) technique of carbon-13 cross-polarization/magic angle spinning (CP/MAS) has been successfully used to obtain high-resolution spectra of whole-thickness, hairy rat skin and to characterize the influence of hydration on the efficiency of cross-polarization and the proton spin-lattice relaxation time in the rotating frame ($T_{1\rho}^{H}$). Spectra obtained with hydrated samples, which were obtained with 50% more accumulations, had comparable signal-to-noise ratio relative to spectra obtained with dried skin, indicating a disordering effect with the presence of water. The integrated area of spectra of low-shifted peaks rose more rapidly with increasing contact time relative to the high-shifted peaks for both hydrated and dried skin. In addition, the carbonyl intensity of the hydrated skin relative to dried skin reached a maximum at shorter times, reflecting an efficient relaxation mechanism of the protons. The shift of the peak maximum to shorter mixing times quantitatively reflects the interaction of the protons of water with the carbonyl moiety.

KEY WORDS: Solid-state nuclear magnetic resonance (NMR); skin; hydration; cross-polarization; magic angle spinning.

INTRODUCTION

The extent of hydration has long been known as being a crucial element in providing the appropriate texture and plasticity for the skin; however, the exact mechanism by which water achieves this effect remains elusive (1). Not unexpectedly, water has been shown to associate with the polar components of the skin, although the interaction has not been sufficiently characterized to allow determination of the mode of binding of water to the skin components (2–5). Related to this question is the role of the stratum corneum lipids in providing the barrier against excess body-water loss and cutaneous permeability (6–11).

Evidence from transport experiments has been collected indicating that hydration has a profound effect on the observed permeability (12,13); however, efforts to characterize the changes induced by the presence of water are hampered by the failure of most techniques when dealing with intact skin. Thus, the feasibility of ^{13}C solid-state NMR has been tested with whole-thickness hairy rat skin. Through the use of cross-polarization, dipolar decoupling, and magic angle spinning (CP/MAS), a high-resolution spectrum was obtained, thereby allowing identification of the chemical constituents giving rise to the individual peaks of the spectra. Moreover, the contact or mixing time was varied to gain insight in the effect of hydration on the skin.

THEORETICAL

Although ^{13}C CP/MAS NMR has been successfully used in many applications including the study of hydration in polymers (14–17), few have used this technique with tissue samples. Obtaining a high-resolution spectrum of solid samples relies on the use of the techniques of cross-polarization, dipolar decoupling, and magic angle spinning (18,19).

Briefly, cross-polarization enhances the signal-to-noise ratio of the normally weak carbon-13 resonant magnetization by using the energy and relaxation properties of the protons. Specifically the protons are brought into a spin-locked condition by a 90° pulse followed by a 90° phase shift of the radiofrequency field (cf. Fig. 1). The carbon-13 nuclei are simultaneously brought into contact with the protons by irradiating the carbons with a radiofrequency field of a strength such that the Hartmann–Hahn condition is satisfied (20):

$$\gamma_H H_{1H} = \gamma_C H_{1C}$$

where γ is the gyromagnetic ratio and H is the field strength. During the contact, magnetization is transferred from the protons to the carbons depending on, among other things, the proximity of the protons to the carbon nuclei. In theory, the rate of transfer has been shown to be dependent on the number of protons attached to the carbons (19). Since the direct relaxation of the carbons in the rotating frame is usually very slow, the rate of decay of the carbon magnetization usually reflects the proton spin-lattice relaxation processes in the rotating frame. The important point is that the decay of the proton magnetization has been shown to be a sensitive indicator of the presence of moisture as is observed in the measured decay of the carbon magnetization (17). The signal from the carbons are then acquired with high-power proton decoupling, which effectively removes the carbon–proton and proton–proton dipolar interactions.

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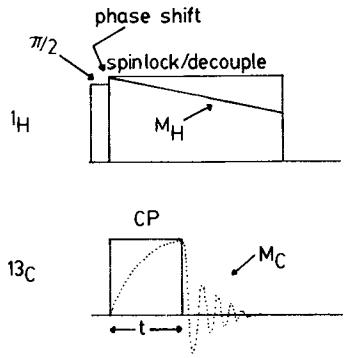

Fig. 1. Typical cross-polarization pulse sequence, where M_H and M_C are the magnetizations of the protons and carbons, and CP refers to the cross-polarization of contact time, t.

The final requirement for obtaining a high-resolution spectrum of a solid sample is magic angle spinning. In the solid state, molecules are no longer undergoing rapid rotational diffusion (18). The result is the appearance of broad lines that have a characteristic pattern due to the dependence of the chemical shift on the angle between the molecular coordinates and the direction of the magnetic field. By rapidly spinning the sample at an angle of 54.7° or the magic angle, the spatial dependence of the chemical shift anisotropy is removed by averaging to the isotropic value. The linewidth is thus effectively reduced to that approaching the linewidth of the solution, although due to residual interactions in the solid, it remains somewhat broadened.

EXPERIMENTAL

Sample Preparation. Whole-thickness skin was obtained from normal, male hairy rats (Sprague–Dawley) that were initially shaved with an electric razor. Rats were sacrificed by carbon dioxide asphyxiation, and subcutaneous fat was removed from the skin by scraping with a razor blade. The skin was dried by placing the samples under a high vacuum (<100 mTorr) overnight and, thereafter, cut into small pieces with a razor blade. The skin was rehydrated by exposing the sample to an atmosphere of 100% humidity as previously reported (2).

NMR Measurements. The ^{13}C CP/MAS spectra were obtained with a 100-MHz (for proton) superconducting magnet and IBM NR/100 AF console. Spin-locked cross-polarization was used to establish the single contact Hartmann–Hahn condition. A delay time of 3 sec was used between successive sampling pulses. For each experiment between 2000 and 3000 scans were accumulated, and phase cycling was used throughout signal acquisition to minimize baseline and intensity artifacts. The samples were spun at the magic angle at a frequency of 3.5 ± 0.5 kHz, which was adjusted by the KBr method (16). The chemical shifts were measured relative to tetramethylsilane using 1,4-di-*t*-butylbenzene as an external reference. Variable contact times were used that ranged from 0.01 to 2 msec. Integrated peak areas obtained from spectra in the absolute intensity mode were used to reflect the carbon magnetization.

RESULTS

Figure 2a illustrates the ^{13}C CP/MAS spectrum obtained for dried skin using a mixing time of 2 msec. The major peaks arise primarily from the proteins found in whole skin, which are predominantly collagen. The peak found at about 170 ppm is due to the common amide carbons along the protein backbone, while the aromatic carbons give rise to the smaller peak at about 130 ppm. The broad distribution of peaks at lower shifts reflects the aliphatic carbons including the α carbons of proline, alanine, glutamine, and leucine at 55 ppm, the α carbon of glycine at 40 ppm, methylene carbons at 30 ppm, and finally, the methyl carbons at about 20 ppm.

In Fig. 2b, the spectrum obtained with hydrated skin is shown. The resolution appears comparable, which indicates a loss in the signal-to-noise since the hydrated sample was acquired with 50% more scans relative to the dried sample with equal sample sizes. Figure 3 is a stacked plot of the spectra of dried and hydrated skin obtained at various contact times, showing the increase in intensity with increasing contact time. In Figure 4, the integrated area of peaks in the range of 170 and 0–85 ppm is given as a function of the contact time for both dried and hydrated skin. The intensity of the peaks at low shifts rise and also decay more rapidly than those at higher shifts in both dried and hydrated skin. Differences in the rate of change of intensity with contact time are seen between dried and hydrated skin for the high-shifted peaks but not for the low-shifted peaks.

DISCUSSION

The presence of hydration appears to reduce the signal-

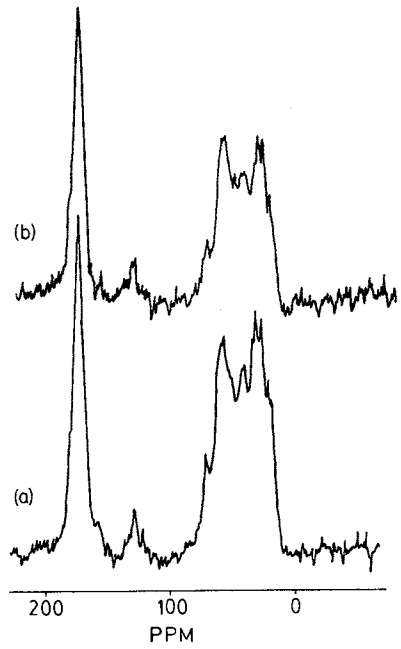

Fig. 2. Comparison of (a) dried and (b) hydrated whole-thickness skin obtained with the cross-polarization sequence and a mixing time of 2 msec. The spectra of the dried sample were obtained with 2000 accumulations, whereas the hydrated sample was obtained with 3000.

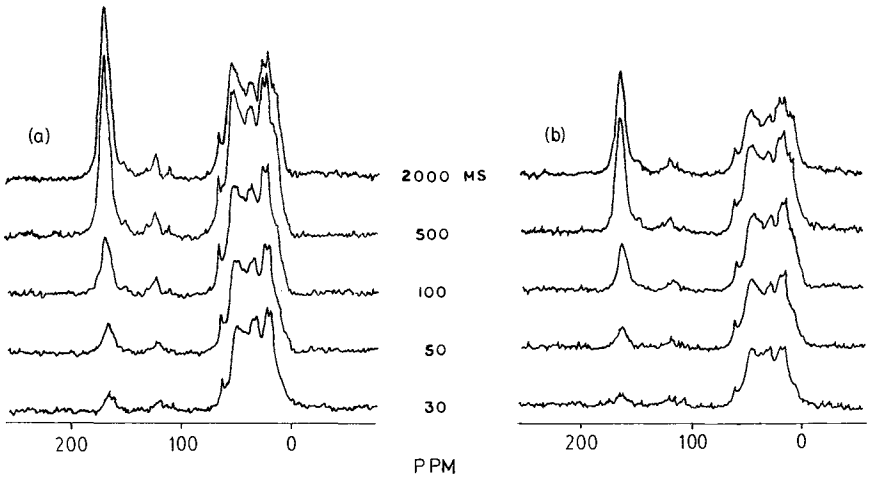

Fig. 3. Spectra for (a) dried and (b) hydrated skin shown as a function of mixing time.

to-noise ratio as seen in Fig. 2b. Anhydrous polymers often yield spectra that appear broadened relative to the spectra obtained with hydrated samples. With the presence of water, there is an apparent relaxation in the noncrystalline regions to a more ordered state, thus resulting in more narrow lines and enhanced resolution (15). Alternatively, the spectra obtained with white spruce wood showed the opposite effect, which perhaps reflects the three-dimensional network of the polymer where the amorphous nature of lignin prevents any relaxation with hydration to a more ordered conformation (17). The latter explanation may also be applicable to the collagen fibrils in the skin, whose three-dimensional array prevent appreciable relaxation of the structure.

The effect of the contact time on the peak intensity of the various peaks in both the dried and the hydrated samples correlates well with the number of the protons attached to the carbons. Thus it is evident that the magnetization transfer is more efficient in carbons that have more attached protons, that is, aliphatic > aromatic > carbonyl (19). This feature of cross-polarization may be exploited for spectral assignments.

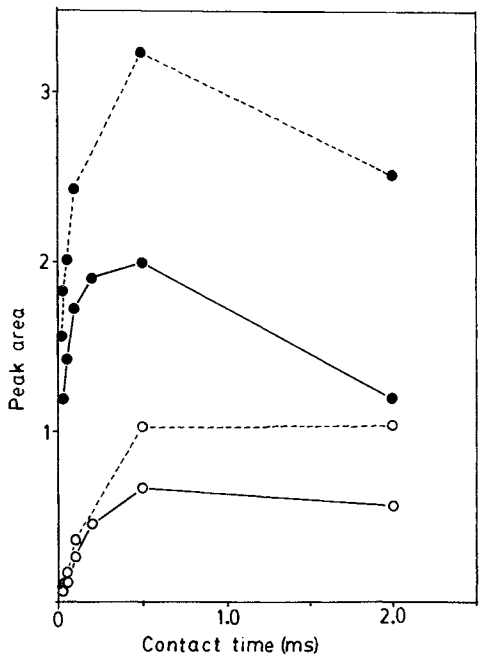

Fig. 4. The integrated areas of the absolute intensity of the carbon nuclei for the peaks in the range of (○) 160–180 ppm and (●) 0–85 ppm for dried (– – –) and hydrated (——) skin.

The terminal decay of the intensity of the carbon magnetization is consistent with the proximity of water to the carbonyl region of the protein backbone. The latter rate of decay generally reflects the magnitude of the proton spin-lattice relaxation time in the rotating frame (18). The major contribution to this parameter depends on the extent of spin diffusion of the protons brought about by the mutal flipping of the proton spins. As would be expected, the presence of water accelerates this process.

The results presented clearly demonstrate the potential usefulness of solid-state NMR and, in particular, cross-polarization with magic angle spinning for analysis of the changes induced by hydration in the structure and dynamics of the skin. Further attempts to analyze the effect of hydration in terms of water-holding capacity or changes in permeability of the stratum corneum are limited since samples of whole-thickness skin were used. In addition to obtaining spectra on isolated sheets of stratum corneum, more work is required to separate better the contributions of the lipid and protein to the observed spectra and gain insight into the structural nature of the skin. Finally, the application of specific pulse sequences is needed for measuring the relaxation times, which may allow analysis of the motions of the individual components.

CONCLUSIONS

The feasibility of ^{13}C CP/MAS NMR for obtaining high-resolution spectra even with complex biological tissue as well as the effect of hydration on whole-thickness hairy rat skin has been quantitatively demonstrated. Further experiments are currently in progress, with the purpose of gaining insight into the effect of hydration on isolated stratum corneum and thereby providing a greater understanding of the role of hydration in the structure and dynamics of the skin.

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