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Chain ordering of *Stratum corneum* lipids investigated by EPR slow-tumbling simulation

K. Nakagawa [a,*], J. Mizushima [b], Y. Takino [c], K. Sakamoto [d,**], H.I. Maibach [e]

[a] *RI Research Center, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan*
[b] *Department of Dermatology, Tokyo Women's Medical University, Tokyo, Japan*
[c] *AminoScience Laboratories, Ajinomoto Co. Inc., Kawasaki, Japan*
[d] *Shiseido Research Center, Yokohama 224-8558, Japan*
[e] *Department of Dermatology, University of California at San Francisco, CA 94143-0989, USA*

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Abstract

We investigated the chain ordering of the lipid bilayer of *Stratum corneum* (SC) using an electron paramagnetic resonance (EPR) spin probe method in conjunction with slow-tumbling simulation. The ordering of SC lipids was evaluated by analysis of the signals of 5-doxylstearic acid (5-DSA) spin probe incorporated into the lamellar lipids. The result obtained with the conventional method of calculating the order parameter using hyperfine values was 0.80. The value of the order parameter obtained by spectral simulation was 0.73. It was found that the conventional method of calculating the chain ordering using hyperfine values could not differentiate subtle EPR spectral changes. However, EPR slow-tumbling simulation can differentiate such subtle spectral changes. Thus, the present EPR investigation suggests that simulation provides more detail about the structure of the lipid bilayer than the conventional method.

Keywords: EPR; Skin lipid; Order parameter; Spin probe; *Stratum corneum*

1. Introduction

Non-invasive spectroscopic characterization of the outermost layer of the skin, *Stratum corneum* (SC), is an important subject in dermatology and cosmetology. The SC is a heterogeneous structure composed of corneocytes embedded in the intercellular lamellar lipid bilayer. It acts as the main epidermal barrier against chemicals, oxidative stress, the UV component of sunlight, and other invasive environmental factors, as well as regulating transepidermal water loss (TEWL) to prevent dehydration of viable cells underneath the SC. The intercellular lamellar lipid bilayers play a critical role in these barrier functions.

At present, no method is available to evaluate precisely the structures and motions of the molecular components of the intercellular lamellar lipid bilayers. TEWL measurement is the only non-invasive method, which indirectly provides knowledge of the status of the lipid layers in relation to water permeation, but the measurement itself does not provide further information regarding the state of the SC. The ordering (or fluidity) of the lipid bilayer can be studied by X-ray analysis only for in vitro SC specimens or model lipid membranes composed of water. Structural information can be obtained non-destructively by analysis of aliphatic spin probes incorporated into the lamellar lipids using electron paramagnetic resonance (EPR) [1–5]. Therefore, EPR can potentially elucidate in detail the skin-lipid structures, as well as their dynamics.

The EPR spin probe method has been utilized to characterize the skin-lipid structures, as well as fluidity [2–5]. The physicochemical properties of intercellular lipids of SC were investigated as a function of various surfactants [2,3], water content [4], and various kinds of spin probes [5]. These studies provided information about the fluidity-related behavior of SC under various conditions by measuring EPR signal intensities and hyperfine values. Recently, we have recognized that the conventional method of calculating the chain ordering using hyperfine values cannot differentiate subtle EPR spectral changes. Changes in the probe behavior are reflected in the EPR line width as well as the line shape, besides the hyperfine values.

* Corresponding author. Tel.: +81 24 547 1111x2823; fax: +81 24 548 3075.
** Corresponding author. Tel.: +81 45 590 6000; fax: +81 45 590 6087.
E-mail addresses: nakagawa@fmu.ac.jp (K. Nakagawa), Kazutami.sakamoto@to.shiseido.co.jp (K. Sakamoto).

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Precise analysis of EPR spectra can be performed by spectral simulation to extract quantitative ordering of the lipid structures [6,7]. Therefore, EPR in conjunction with a simulation method should be useful to obtain detailed information regarding skin-lipid structures. Such knowledge of the SC structural changes and mobility would be important in understanding the mechanism of irritant dermatitis and other SC diseases.

In this paper, we describe an ex vivo EPR investigation of the chain ordering of SC using an aliphatic spin probe together with slow-tumbling simulation. EPR spectra were analyzed both by conventional calculation and slow-tumbling simulation. The obtained values of the order parameter are discussed in terms of EPR spectral changes as well as hyperfine coupling values.

2. Experimental

2.1. Sample preparations of stripped SC from volunteers and spin labeling of the SC for ex vivo measurements

The SC was removed from the mid-volar forearm of volunteers, who had given informed consent to the procedure, with a single drop of cyanoacryrate resin, and placed on quartz glass (5 mm × 15 mm; Nihon Denshi, Tokyo, Japan). The spin probe labeling agent, 5-doxylstearic acid (5-DSA), was purchased from Aldrich–Sigma Chemical Co. Inc. and used as received. The SC samples were incubated in a 1.0 mg/dl 5-DSA aqueous solution for 30–60 min at 37 °C. After rinsing with deionized water to remove excess spin probe, the SC sample was mounted in the EPR cavity. The sample preparation has been described in detail elsewhere [1–3].

2.2. Cadaver-SC preparation and spin-labeling of the SC for in vitro measurements

Human cadaver skin, excised from the abdomen within 24 h of death, and dermatomed to a thickness of approximately 500 μm, was obtained from Northern California Transplant Bank (San Rafael, CA, USA) [2,3]. Epidermis was separated from dermis by immersing the skin in a water bath set at 60 °C for 2 min, followed by mechanical removal. Then the epidermis was placed cadaver-SC side up on filter paper and floated on 0.5 wt% trypsin (type II; Sigma) in phosphate-buffered saline (PBS; pH 7.4) for 2 h at 37 °C. After incubation, any softened epidermis was removed by mild agitation of the SC sheet, which was then dried and stored at −20 °C.

A slice of the dried SC sheet (ca. 0.5 cm^2; 0.7 cm × 0.7 cm) was incubated in 1.0 mg/dl 5-DSA aqueous solution for 30–60 min at 37 °C, and then washed with deionized water. The labeling time for control EPR spectra is defined as 0 h (zero) (i.e., measurement was done immediately after incubation).

2.3. EPR measurements

A commercially available X-band (9 GHz) EPR spectrometer was used to measure the fluidity of the SC samples. The typical spectrometer settings were as followings: microwave power, 20 mW; time constant, 0.1 s; sweep time, 240 s; modulation, 0.1 mT; sweep width, 12.5 mT. All measurements were performed at ambient temperature. Then, the obtained EPR spectra of 5-DSA in the various SC samples were analyzed using two methods: conventional calculation of the order parameter and spectral simulation [6].

2.4. Conventional EPR anaysis

The inclination of the principal axis of the nitroxide radical to the rotational axis of the long-chain probe molecule represents a measure of the order–disorder of the molecular assemblies of a membrane. The order parameter indicates the membrane chain dynamics and microenvironment of the medium in which the spin probe is incorporated. The conventional order parameter (S) is determined from the hyperfine coupling of the EPR signals according to the following relations [8]:

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{ZZ} - \frac{1}{2}(A_{XX} + A_{YY})} \frac{a}{a'}, \quad (1)$$

$$a' = \frac{A_{\parallel} + 2A_{\perp}}{3}, \quad (2)$$

where a is the isotropic hyperfine value $(A_{XX}+A_{YY}+A_{ZZ})/3$; A_{XX}, A_{YY}, and A_{ZZ} are the principal values of the spin probe. In calculation based on the experimental spectra, the following principal components were used for 5-DSA [9]:

$A_{XX}, A_{YY}, A_{ZZ} = (0.66, 0.55, 3.45)$ mT

$g_{XX}, g_{YY}, g_{ZZ} = (2.0086, 2.0063, 2.0025)$

The experimental hyperfine couplings of $2A_{\parallel}$ and $2A_{\perp}$ are obtained from the EPR spectrum as shown in Fig. 1. The order parameter indicates that the S value increases with increasing

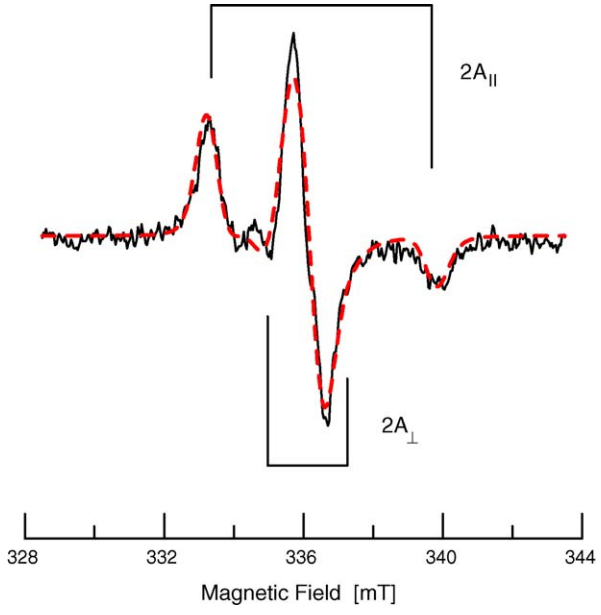

Fig. 1. The ex vivo experimental (solid line) and simulated (dotted line) EPR spectra of 5-DSA in stripped SC from human mid-volar forearm. Parallel and perpendicular hyperfine components are indicated for the conventional calculation.