Microchemical Journal 117 (2014) 183–186

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Protein structure is changed in psoriatic skin on the unaffected region — Imaging possibility with ATR-FTIR spectroscopy

Boglárka Balázs [a,b], Gabriella Farkas [a], Ottó Berkesi [c], Rolland Gyulai [d], Szilvia Berkó [a], Mária Budai-Szűcs [a], Piroska Szabó-Révész [a], Lajos Kemény [e], Erzsébet Csányi [a,*]

[a] Department of Pharmaceutical Technology, University of Szeged, Eötvös utca 6., H-6720 Szeged, Hungary
[b] Gedeon Richter Ltd., Gyömrői út 17-19., H-1103 Budapest, Hungary
[c] Department of Physical Chemistry and Materials Science, University of Szeged, Aradi Vt. 1., H-6720 Szeged, Hungary
[d] Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs, Kodály Z. u. 20., H-7624 Pécs, Hungary
[e] Department of Dermatology and Allergology, University of Szeged, Korányi fasor 6., H-6720 Szeged, Hungary

ARTICLE INFO

Article history:
Received 8 May 2014
Accepted 2 July 2014
Available online 9 July 2014

Keywords:
ATR-FTIR spectroscopy
Psoriatic skin
Protein structure
Amide-I band
Fourier self-deconvolution

ABSTRACT

Psoriasis is a T lymphocyte-mediated inflammatory disorder that affects the skin. A number of studies have demonstrated the occurrence of lipid alterations in psoriatic skin, resulting in a highly perturbed stratum corneum (SC). Relatively little attention has been paid to the protein conformation of the SC. In this study, the attenuated total reflection Fourier transform infrared (ATR-FTIR) spectrum of the untreated psoriatic patients' unharmed SC was obtained after tape stripping. We focused on the amide-I band components in order to establish whether there are any protein alterations in the intact areas of psoriatic skin. Fourier self-deconvolution (FSD) of the amide-I band was followed by curve-fitting to generate the underlying components. Integration of band areas provided an estimate of the secondary structure. The results indicated decreases in all amide-I band components, the peak at 1660 cm^{-1} revealing the most dramatic change. This peak is characteristic of the turn structure in the protein chain. The decrease is marked in the case of the β-sheet structure at 1630 cm^{-1} too. This ATR-FTIR imaging is a rapid and simple noninvasive method, promotes a better understanding of the disease, and would be helpful in following the treatment.

1. Introduction

Psoriasis, one of the most common chronic inflammatory skin diseases, with a frequency of approximately 1–2% of the population in the developed world, is characterized by regular exacerbations of cutaneous symptoms and frequent articular involvement. The prototypic psoriatic skin lesions are sharply demarcated erythematous plaques covered by silvery-white scaling, most often seen on the elbows, knees, scalp, umbilicus and lumbar area. The diagnosis is usually made on clinical grounds, though rarely a skin biopsy is necessary. The disease has a polygenic inheritance, and the expression of the symptoms is greatly influenced by environmental factors, such as stress or streptococcal infections [1,2].

Histologically, the disease is characterized by keratinocyte hyperproliferation, lymphocyte and granulocyte infiltration of the epidermis and dermis, and vasodilation of the dermal blood vessels. Hyperproliferation of the keratinocytes results in highly perturbed functions of the stratum corneum (SC). The SC, the uppermost layer of the epidermis, consists of keratin-rich anucleate nonviable cells (corneocytes) embedded in an intercellular matrix of lipids [3]. It is well known that multiple lipid bilayers play an important role in the permeability of the skin, and psoriatic skin is more permeable than healthy skin [2]. A number of studies have demonstrated that psoriatic skin exhibits alterations in lipid metabolism [4,5], whereas the changes in the proteins and their conformations in the SC of psoriatic skin have not been well studied to date.

One of the most common applications of Fourier transform infrared (FTIR) spectroscopy in protein studies is the analysis of secondary structure. FTIR spectroscopy involves the measurement of the wavelength and intensity of the absorption of IR radiation by a sample. The amide-I band region (1700–1600 cm^{-1}) is the spectral region most sensitive to the secondary structural components of the proteins; this is due very largely (approximately 80%) to the C═O stretch vibrations of the peptide linkages. Fourier self-deconvolution (FSD), a band-narrowing method, has not only enriched the qualitative interpretation of IR spectra, but has also provided a basis for the semiquantitative estimation of the secondary structure of proteins [6,7].

Attenuated total reflection FTIR (ATR-FTIR) spectroscopy is a tool that is often used to characterize the lipids, proteins and water content of the SC [8]. The sample is placed on an IR-transparent crystal, the IR

* Corresponding author at: Department of Pharmaceutical Technology, University of Szeged, Eötvös str. 6, H-6720 Szeged, Hungary. Tel.: +36 62 545 573; fax: +36 62 545 571.
E-mail address: csanyi@pharm.u-szeged.hu (E. Csányi).

http://dx.doi.org/10.1016/j.microc.2014.07.003
0026-265X/

beam is directed onto the crystal with high refractive index and the internal reflectance creates an evanescent wave which penetrates into the sample. Unlike the case of the KBr pellet method, this method does not require sample preparation. We made use of the application of an adhesive tape to obtain the spectrum of human SC in vivo [9].

The aim of the present paper is to give an account of the primary results of an in vivo study of intact psoriatic skin, using the components of the amide-I band to determine the changes in the secondary structure of the proteins by combining tape stripping and ATR-FTIR spectroscopy.

2. Materials and methods

2.1. Experiments

Psoriatic SC samples were obtained from 6 untreated psoriatic patients (3 males and 3 females) aged between 32 and 64. Normal SC samples were collected from 20 healthy volunteers (10 males and 10 females) aged between 21 and 42 with no history of dermatological disease. The study was approved by the local ethics committee (Government Office of Csongrád County Policy Administration Services of Public Health, PSO-SPEKT-001). All volunteers were asked not to apply any moisturizer or cosmetic product for at least 24 h before the process. The forearm skin of each volunteer was stripped with adhesive cellophane tape; this was repeated up to 25 strips. In the case of the psoriatic patients, the samples were taken from the unharmed skin of the forearm. Every first tape with one strip was discarded because of the possibility of surface contamination. Every second adhesive tape with three strips was analyzed, because this gave the most intensive IR spectrum.

2.2. FTIR spectroscopy and curve-fitting analysis

ATR-FTIR spectra were recorded with an Avatar 330 FT-IR spectrometer (Thermo Nicolet, USA) equipped with a horizontal ATR crystal (ZnSe, 45°), between 4000 and 400 cm^{-1}, at an optical resolution of 4 cm^{-1}. 128 scans were co-added and all spectral manipulations were performed by using Thermo Scientific's GRAMS/AI Suite software. The components of the amide-I band and their relative intensities were estimated semiquantitatively in the 1695–1600 cm^{-1} region of the FTIR spectra by a curve-fitting algorithm using Gaussian–Lorenzian mixing functions. The best fits were found by an iterative process minimizing the standard error.

2.3. Statistical analysis

The results of curve-fitting were evaluated and analyzed statistically by Student's t-test. The data given are the averages of the results of 13 parallel (3 psoriatic and 10 healthy) experiments ± SD ($p < 0.05^*$, $p < 0.01^{**}$).

3. Results and discussion

The amide-I band appears clearly in the ATR-FTIR spectrum of the skin as a consequence of the coupling of the transition dipole moments of the amide groups within the protein backbone. Various protein secondary structures have their own characteristic amide-I bands. However, because of the strong overlap between the characteristic bands, the amide-I bands of the recorded infrared spectra are too complex to permit the direct determination of the components with various secondary structures [10].

Band-narrowing procedures such as FSD and curve-fitting greatly enhance the potential of this band as a meaningful structural probe. Such mathematical processes increase the degree of separation by narrowing the half-bandwidths of the individual components for easier visualization without seriously distorting the spectrum. The main disadvantage of this procedure is its tendency to magnify both the signal and the noise in the spectrum. This causes a dramatic increase in the noise level, which necessitates coupling the FSD with a smoothing function [6,11,12].

FSD of the corresponding ranges of the recorded spectra were used to determine the approximate frequencies of the components of the amide-I bands indicating the presence of protein chain sections with various secondary structures [10,13]. Seven bands were fitted to all the spectra and their areas were compared (Fig. 1). The ratios of the integrated band areas provide an estimate of the proportions of the parts with various secondary protein structures.

A protein molecule is formed from a chain of amino acids. The secondary structure of a protein is determined by the set of dihedral angles (φ, ψ) which define the spatial orientation of the peptide backbone, and the presence of specific hydrogen-bonds. Regular secondary structures include α-helices and β-sheets. An ideal α-helix has 3.6 residues per turn, and is built up from a contiguous amino acid segment via backbone–backbone hydrogen-bond formation between amino acids in positions i and i + 4. The residues taking part in an α-helix have φ angles around −60° and ψ angles around −45°.

β-Sheets are created when atoms of β-strands are hydrogen-bonded. β-Sheets may consist of parallel strands, antiparallel strands or a mixture of parallel and antiparallel strands. In parallel β-sheets the strands run in one direction, whereas in antiparallel sheets they run in alternating directions. The dihedral angles of the β-sheet are φ ~ −130° and ψ ~ −120°, forming an extended structure with some right-handed twist.

Turns allow a protein to fold back on itself and are stabilized by a hydrogen-bond that holds the ends together. They are classified according to the number of residues involved in the hydrogen-bonded structure. Unordered structure is generally defined as a conformation that is not helix, sheet or turn [13].

In the SC, greater flexibility is achieved through loose packing of the keratin filaments and a lower number of disulfide crosslinks. It was observed that the overall intensities of the amide-I bands were lower for the psoriatic SC than for the healthy SC (Fig. 2), but the peak at 1660 cm^{-1} exhibited the most pronounced alteration, as shown in Fig. 3. The proteins in the SC are mainly in α-helical conformation [14]. The peak at 1650 cm^{-1} indicates the presence of an α-helix (Fig. 3), which always has the largest area. We focused on the peak at 1660 cm^{-1}, characteristic of the turns in the protein chain [13]. The intensity of this peak decreases almost to zero in psoriatic patients, indicating the drop in concentration in the turn protein regions (Table 1). We have given the results for men and women separately, because of the differences in skin between the genders [15].

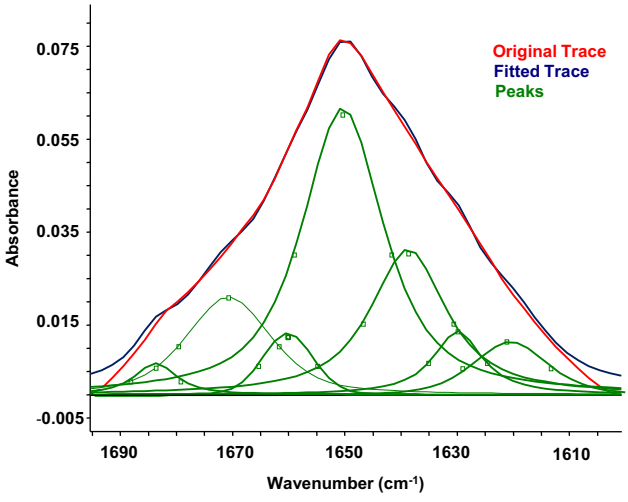

Fig. 1. The original FTIR spectra of the SC and the deconvolved and curve-fitted components.