

Structure of Water, Proteins, and Lipids in Intact Human Skin, Hair, and Nail

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Raman spectroscopy is a nondestructive analytical method for determining the structure and conformation of molecular compounds. It does not require sample preparation or pretreatment. Recently, near-infrared Fourier transform Raman spectroscopy has emerged as being specially suited for investigations of biologic material. In this study, we obtained near-infrared Fourier transform Raman spectra of intact human skin, hair, nail, and stratum corneum. We disclosed major spectral differences in conformational behavior of lipids and proteins between normal skin, hair, and nail. The amide I and III band location indicated that the majority of proteins in all samples have the same secondary α -helix structure. Positions of (S-S) stretching bands of proteins revealed a higher stability of the disulfide bonds in the hair and the nail. Analysis of vibrations of protein -CH

groups showed that in the hair and the nail the proteins are apparently highly folded, interacting with the surroundings only to a small degree. The position of lipid specific peaks in spectra of hair, nail, and stratum corneum suggested a highly ordered, lamellar crystalline lipid structure. A greater lipid fluidity was found in whole skin. Assessment of the structure of water clusters revealed that mainly bound water is present in the human skin, stratum corneum, and nail. In conclusion, structural changes of water, proteins, and lipids in intact skin and skin appendages may be analyzed by Raman spectroscopy. This technique may be used in the future in a noninvasive analysis of structural changes in molecular compounds in the skin, hair, and nail associated with different dermatologic diseases. **Key words:** Raman spectroscopy/stratum corneum/vibrational spectroscopy. *J Invest Dermatol* 110:393-398, 1998

The fundamental role of skin is to protect the underlying tissues from undesirable environmental factors and to prevent excessive loss of fluids from the body. The barrier function is mainly achieved due to the unique structure of proteins and lipids in the skin. For example, the very high stability of the hair and the nail is due to stiffness of the central α -helical core of keratin and a high number of disulfide crosslinks. In contrast, in the stratum corneum the number of these crosslinks is lower and the greater flexibility is achieved due to loose packing of keratin filaments, allowing for a significant degree of side-chain mobility (Mack *et al*, 1988). The lipids in the stratum corneum are arranged into lamellar structures that are covalently attached to proteins of cornified envelopes. This allows the lipids to form a water-impermeable layer in the stratum corneum (Imokawa *et al*, 1991; Lazo *et al*, 1995). Water itself, both in the stratum corneum and in the whole skin, allows for plasticity and barrier function (Blank, 1952).

Despite a considerable progress due to the introduction of modern analytical techniques such as X-ray and neutron scattering, electron-spin resonance of spin labels, and nuclear magnetic resonance, the detailed molecular structure of proteins, lipids, and water in the skin has not been completely elucidated. There is still an ongoing debate

whether the proteins in the stratum corneum are primarily in the α -helical or β -sheet conformation (Garson *et al*, 1991; Leveque, 1994). Little is known about the structure of the lipids. The water content in stratum corneum has been previously studied (Elsner *et al*, 1994); however, little is known about the water structure in the whole skin, the hair, and the nail. These issues have been difficult to resolve because most of the methods used for the investigation of the molecular structure require purification of the compound of interest or sample processing, procedures that are likely to alter the conformation of the molecules.

Near-infrared Fourier transform (NIR-FT) Raman spectroscopy that has recently been introduced in biomedical research, allows the study of the molecular conformation of the compounds in their natural microenvironment (Liu *et al*, 1992; Mizuno *et al*, 1994; Frank *et al*, 1995). NIR-FT Raman spectroscopy exploits an effect in which a small portion of monochromatic light scattered by a substance has a frequency that is different from that of the incoming beam by an amount equal to the vibration frequency of the chemical bond. Frequency shifts of the scattered light can be analyzed and presented as spectra. Spectral bands represent vibrations characteristic for chemical bonds within the molecules of the examined sample. The major advantages of NIR-FT Raman spectroscopy are that it can be used for very complex unpurified samples, it requires only minute quantities of the compound, and it is applicable to biomolecules in any state of aggregation. Raman spectroscopy detects the molecular vibrations at $\approx 10^{-12}$ s; it is therefore able to directly detect hydrogen bond vibrations. Because hydrogen bonds are the major bindings responsible for the intermolecular structure and properties of many compounds, Raman spectroscopy can be used for the direct assessment of the structure of water, proteins, and lipids (Pimentel and McClellan, 1960; Faurskov Nielsen, 1979, 1993, 1997; Krishnamurthy *et al*, 1983; Tu, 1986;

Manuscript received September 24, 1997; accepted for publication November 24, 1997.

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Abbreviations: NIR-FT, near-infrared Fourier transform; δ , deformational vibrations; ν , stretching vibrations; ν_s , symmetric stretching vibrations; ν_{as} , asymmetric stretching vibrations.

Miura and Thomas, 1995; Colainni *et al*, 1995). In contrast, slower spectroscopic techniques like nuclear magnetic resonance, measuring at a time scale corresponding to microseconds, give only an estimate of an average of molecular motions. Structural changes detected by Raman spectroscopy, caused by alterations of hydrogen bonding, are likely to be of biologic importance (Faurskov Nielsen *et al*, 1982a, b; Tu, 1986; Miura and Thomas, 1995; Colainni *et al*, 1995; Faurskov Nielsen, 1997).

Pioneering work on NIR-FT Raman spectroscopy of the skin, hair, and nail has been carried out by Barry *et al* (1992) and Williams *et al* (1994). These authors were the first to assign the Raman spectral bands to specific vibrational modes of chemical bonds in proteins and lipids in the stratum corneum. Importantly, they showed differences in Raman spectra between normal and diseased skin, thus opening the possibility of using NIR-FT Raman spectroscopy for noninvasive dermatologic diagnosis (Edwards *et al*, 1995). Just recently, alterations in the molecular structure of proteins and lipids in benign and malignant skin tumors have been described by Raman spectroscopy (Gniadecka *et al*, 1997a, b).

Here, we employed NIR-FT Raman spectroscopy to investigate the molecular conformation of proteins, lipids, and water in human stratum corneum, hair, nail, and the whole skin. The results allowed us to make correlations between the molecular structure and the macroscopic properties of these integument tissues.

MATERIALS AND METHODS

Skin, hair, and nail samples Three millimeter punch biopsies of normal skin (buttock, lower leg, back, arm) were collected from 44 healthy individuals (age 18–35, 28 men, 16 women) with skin types I–IV. The samples were kept at 4°C in a humid environment and analyzed within 30 min. Samples of the hair, nails, and stratum corneum (scraped from the arm skin with a scalpel) were obtained from 10 individuals. No sample pretreatment of any kind was performed. The protocol was accepted by the Ethics Committee of Copenhagen, Denmark.

Raman spectroscopy A Bruker (Karlsruhe, Germany) IFS 66 interferometer equipped with a FRA 106 module or a 100 FRA-Raman spectrometer was used. The 1064 line at 300 mW from a Nd:YAG laser was the excitation source. A liquid nitrogen-cooled Ge detector was used. The laser beam was focused to a spot of $\approx 100 \mu\text{m}$ in diameter on the epidermal site of the skin biopsy, on the stratum corneum, the nail, or the hair sample, which during the procedure were placed in a stainless steel cup. Two hundred and fifty scans at a 4 cm^{-1} spectral resolution were collected from each sample. The total registration time was 10 min. The radiation scattered by the sample contains the frequency of the incident radiation (Rayleigh line) and several lines of other frequencies characteristic of the molecular vibrations (Raman lines). Frequency shifts of Raman lines relative to the exciting line are presented in the spectrum. The spectral range of the acquired spectra was from 0 to 3500 cm^{-1} (Fig 1). OPUS software (Bruker) was used to evaluate spectral characteristics. The original OPUS format was transferred to ASCII format and analyzed by the Matlab (S. Natick, MA) program. No normalization of any particular spectral band was performed but all spectra were scaled by subtraction of the mean and division by the standard deviation, which is a standard procedure to facilitate graphical presentation of the data (Gemperline *et al*, 1990; Borggard and Thodberg, 1992). Smoothing procedures were not used. Background correction for instrumental response has been performed as previously described (Colainni *et al*, 1995; Gniadecka *et al*, 1997a). The correction of the spectra is particular important in the regions above 3000 cm^{-1} , where the detector sensitivity drops rapidly off, and in the low frequency region due to the effect of the filters. For assessment of water content, however, the intensities (*I*) of the peaks at 3250 cm^{-1} and 2940 cm^{-1} (I_{3245}/I_{2940}) were estimated in the raw spectra, because of the clearer shape of the water peak in nonbackground corrected spectra.

The low frequency region (10–300 cm^{-1}) of the Raman spectra of molecules is difficult to study due to the high intensity of the Rayleigh line. Therefore the low frequency spectra can be conveniently studied in the R(*v*) representation (Faurskov Nielsen, 1993, 1997), where the Rayleigh line is converted into a plateau:

$$R(v) = v[1 - \exp(-hc\nu/kT)]I(v) \quad (1)$$

where *I*(*v*) is the intensity in the Raman spectrum at a Raman shift on *v* cm^{-1} , *c* is the velocity of light, *T* is the absolute temperature, *h* is Planck's constant, and *k* is Boltzmann's constant.

In lipids the ratio of the symmetric methylene C–H stretching modes intensities (peak height), I_{2890}/I_{2850} , is sensitive to the packing order of the acyl

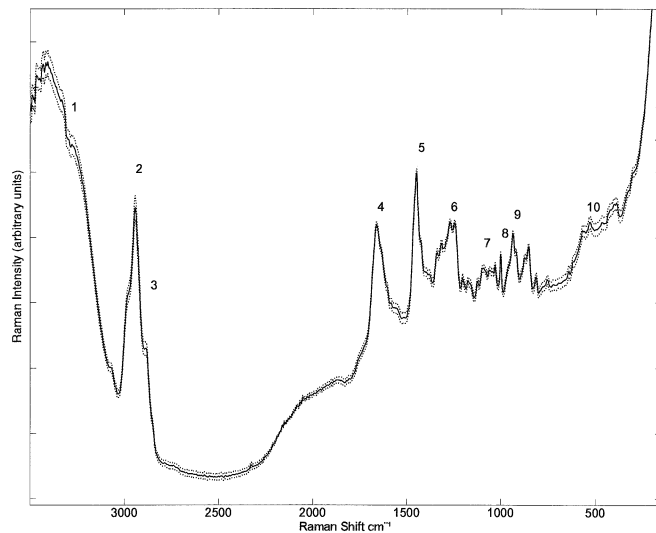


Figure 1. NIR-FT Raman spectra of the intact skin over the range 180–3500 cm^{-1} . Raman spectra were obtained without any previous sample pretreatment: 1.064 μm excitation, 4 cm^{-1} spectral resolution, 250 scans. Background corrected spectra (as described in *Material and Methods*) of skin samples obtained from 44 healthy volunteers with skin types I–IV. —, mean values; ·····, 95% confidence intervals. The following peaks were identified: 1, $\nu(\text{OH})$ in water; 2, $\nu_{\text{as}}(\text{CH}_3)$ in proteins; 3, $\nu_{\text{s}}(\text{CH}_2)$ in lipids; 4, amide I band of proteins; 5, $\delta(\text{CH}_2)(\text{CH}_3)$ in proteins and lipids; 6, amide III band; 7, bands determining fluidity of phospholipids (determined by the appearance of peaks around 1060 cm^{-1} , 1100 cm^{-1} , and 1130 cm^{-1}); 8, phenyl ring $\nu(\text{C}=\text{C})$ breathing mode; 9, $\nu(\text{C}=\text{C})$ band in proteins; 10, $\nu(\text{S}=\text{S})$ in proteins; 11, tetrahedrally coordinated water structure.

chains (Gaber and Peticolas, 1977). The order parameter for the lateral interaction, S_{lat} , has been developed:

$$S_{\text{lat}} = [I_{\text{CH}_2}(\text{sample}) - 0.7]/1.5 \quad (2)$$

where I_{CH_2} is the intensity of the 2890 cm^{-1} band divided by the intensity of the 2850 cm^{-1} band.

$S_{\text{lat}} = 1$ when a phospholipid is in the crystal state ($I_{\text{CH}_2} = 2.2$), and $S_{\text{lat}} = 0$ when a phospholipid is in the liquid state ($I_{\text{CH}_2} = 0.7$). This parameter reflects the intermolecular structure of lipids and decreases in the following order: lamellar liquid, hexagonal or cubic liquid crystal, micellar solution, and solution in organic solvent (Gaber and Peticolas, 1977).

The ratio of intensities of CH (around 2930 cm^{-1}) and OH stretching bands (around 3250 cm^{-1}) is related to hydrogen bonding or hydration between protein and water (Cavotorta *et al*, 1976; Samanta and Walrafen, 1978). Therefore, we determined skin hydration (water content) by calculating I_{2940}/I_{3250} [intensity (peak height) of the 2940 cm^{-1} band/intensity of 3250 cm^{-1} band].

RESULTS

Skin NIR-FT Raman spectra were highly reproducible (Fig 1). Only minor differences occurred in spectra of different skin types. Spectra of pigmented skin (skin types III and IV) and of dark hair showed elevation in the region 1800–2300 cm^{-1} , which is due to the fluorescent background arising from increased pigmentation. The major protein, lipid, and water peaks, however, showed no skin type dependent changes in the shape or in the position of the bands.

Proteins in whole skin, stratum corneum, hair, and nail are in the α -helix conformation Significant Raman vibrations sensitive for protein conformation are those of the peptide bonds (Fig 2a). Among seven important vibrations, two – the amide I and amide III vibrations (Tu, 1982, 1986) – are clearly represented in Raman spectra of the skin. Amide I mainly reflects the C=O stretching vibrations, whereas for amide III the band is a more complex mode involving several chemical bonds (Fig 2a) (Tu, 1986).

Combining frequencies of amide I and III intensity maxima (Table I) indicated that the majority of the proteins in the whole skin, stratum corneum, hair, and nail were in the α -helix conformation (Tu, 1986) (Fig 3a). Moreover, a strong C–C stretch band at $\approx 935 \text{ cm}^{-1}$, which

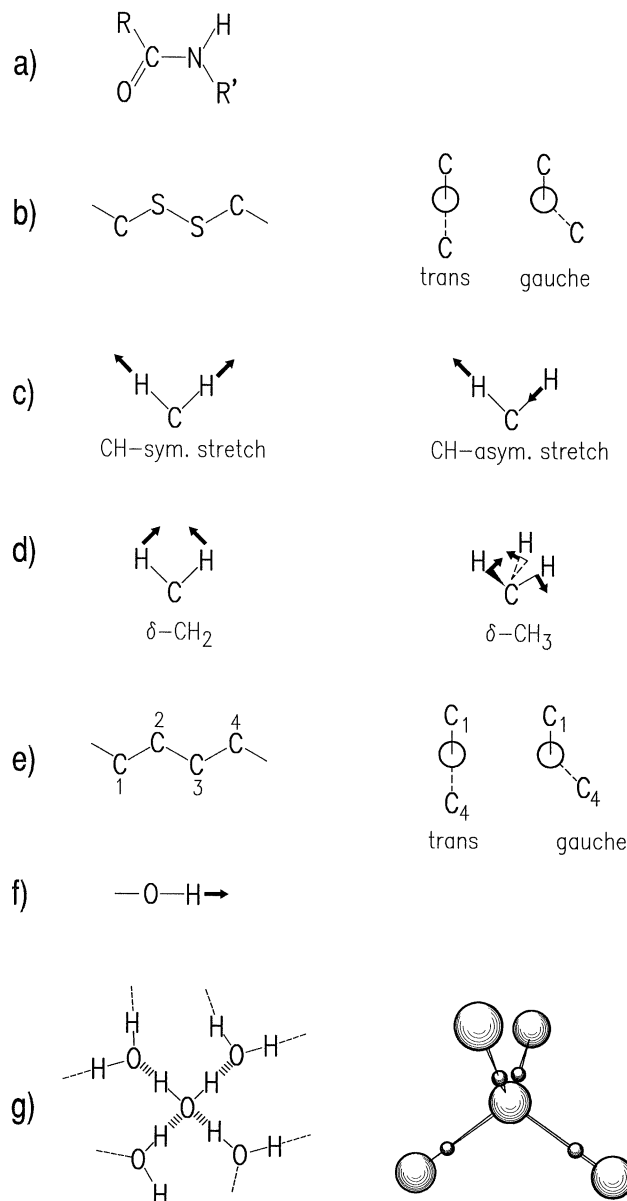


Figure 2. Schematic presentation of molecular vibrations in proteins, lipids, and water. (a) Peptide bond in proteins. The amide I involves mainly a stretching of the C=O bond. The amide III mode is more complex, involving a stretch of the C-R and C-N bonds together with a deformation of the CNH angle. (b) An illustration of trans- and gauche- conformations. These conformations occur for the C-S, S-S, and S-C bonds. (c) C-H stretching vibrations, symmetrical and asymmetrical. (d) $\delta(\text{CH}_2)(\text{CH}_3)$ vibrations. (e) Acyl back bone chain in trans (1) and gauche (2) conformation. (f) O-H stretching in water. (g) Hydrogen bonding in tetrahedral water structure.

is typical for α -helix, was invariably present in the spectra (Frushour and Koenig, 1974, 1975; Barry *et al.*, 1992).

The disulfide stretch in proteins has the most energetically favorable conformation in the hair and nail The disulfide S-S stretch was visible in the region 500–550 cm^{-1} . Depending on the conformation of the C-S-S-C atoms (Tu, 1982; Lazo *et al.*, 1995), the following Raman bands have been identified in natural proteins: the most common and energetically favorable *gauche-gauche-gauche* conformation ($\approx 510 \text{ cm}^{-1}$), and the less stable *gauche-gauche-trans* ($\approx 525 \text{ cm}^{-1}$) and *trans-gauche-trans* (540 cm^{-1}) (Fig 2b). In the spectra of the hair and the nail, the S-S band was prominent and was located at around 510 cm^{-1} (Fig 3b), indicating that these bonds are primarily in the *gauche-gauche-gauche* conformation; however, a shoulder at 520 cm^{-1} indicated that some proportion of S-S bonds are in the

gauche-gauche-trans conformation (Fig 2b). The position of the S-S band in stratum corneum in our spectra around 520 cm^{-1} suggests a less stable conformation, *gauche-gauche-trans*, of protein in stratum corneum. In normal skin, the S-S band was found at 540 cm^{-1} (Table I, Fig 3b), which implies *trans-gauche-trans* conformations (Fig 2b), and are thus probably more unstable (Sugeta and Miyazawa, 1972, 1973; Nash *et al.*, 1985; Weiss-Lopez *et al.*, 1986).

Proteins of hair and nail are highly folded The CH stretching vibrations of the protein are represented in the region 2800–3000 cm^{-1} . The 2890 cm^{-1} and 2850 cm^{-1} bands originate from the lipids (see below) whereas the bands 2980 cm^{-1} , 2930 cm^{-1} (both originate from the CH_3 asymmetric vibrations), and 2900 cm^{-1} (CH_2 symmetric vibrations) are protein specific (Fig 2c) (Tu, 1986). Usually, the CH_3 asymmetric line (2930 cm^{-1}) was most prominent in the spectra, and the CH_3 asymmetric band (2980 cm^{-1}) formed a shoulder (Fig 3c). The 2900 cm^{-1} line was only occasionally resolved in our spectra. As shown in Table I, the mean wave numbers of the CH_3 asymmetric line were significantly higher in the spectra of whole skin than in the hair and nail or stratum corneum. Based on the investigations on Rnase (Verma and Wallach, 1977a, b), it is probable that a shift of the asymmetric CH_3 line towards higher wave numbers (Fig 3c) represents unfolding of the protein and exposure of its aliphatic amino acid chains to the surrounding water. Thus, in hair and nail the protein is apparently highly folded, interacting with its surroundings to only a minimal degree.

The band at 1425–1453 cm^{-1} represents vibrations of $\delta(\text{CH}_2)$ scissoring of lipids and $\delta(\text{CH}_2)\delta(\text{CH}_3)$ of proteins (Barry *et al.*, 1992; Gniadecka *et al.*, 1997b) (Fig 2d). This band was present at $\approx 1447 \text{ cm}^{-1}$ in the stratum corneum, the hair, and the nail spectra and at higher wave numbers at $\approx 1451 \text{ cm}^{-1}$ in the whole skin (Fig 3d).

Higher order in the hair, nail, and stratum corneum than in the whole skin Lipids are present in several conformations. With transition from the crystal to the solution, the fluidity of the lipid increases and the order of the lateral packing of the acyl chains decreases so that the lipid structure becomes more chaotic. The fluidity of the lipid, and thus its intramolecular conformation, can be assessed using the intensity of the bands in the region 1000–1150 cm^{-1} (Lippert and Peticolas, 1971; Susi *et al.*, 1980; Akhtar and Edwards, 1997). In the crystal state, all chains are in the all-*trans* conformation represented by the bands 1066 cm^{-1} and 1130 cm^{-1} (Fig 2e). Lipid melting causes a transition to a more chaotic *gauche* conformation characterized by the disappearance of the 1066 cm^{-1} and 1130 cm^{-1} peaks and the emergence of a 1100 cm^{-1} peak. In the spectra of nail, hair, and stratum corneum the 1130 cm^{-1} and 1030 cm^{-1} peaks were most prominent, whereas in the whole skin the 1100 cm^{-1} peak dominated (Table I, Fig 3e). This implies that lipid structure is highly ordered in hair, nail, and stratum corneum, whereas the fluidity of lipids is greater in the whole skin. Further information on lipid structure can be gathered by examining the regions of CH group vibrations at 2850–2890 cm^{-1} (Fig 3c). The S_{lat} ratio (eqn 2) is proportional to the degree of the lateral packing of the acyl chains (Gaber and Peticolas, 1977; Wegener *et al.*, 1996). In hair, nail, and stratum corneum the S_{lat} value was 1, suggesting that the majority of the lipids are in the crystal state (probably lamellar liquid crystal). It also implies a very low degree of intermolecular interactions between lipids and proteins (Larsson and Rand, 1973). In contrast, for the whole skin the S_{lat} value was 0.4, implying that lipids lose their crystalline structure and interact more freely with the proteins in their microenvironment (Lis *et al.*, 1976a, b).

Water in the whole skin is present mainly in the bound form The intramolecular vibrations of hydrogen bonded water include two OH stretching bands in the 2800–3400 cm^{-1} region, and the bending band near 1645 cm^{-1} (Maeda and Kitano, 1995; Walrafen and Chu, 1995). The bending mode is weaker in intensity than the stretching bands, and therefore it is hidden by the amide I vibrations around 1650 cm^{-1} . The symmetric OH stretching is found around 3250 cm^{-1} (Fig 2f), whereas the Raman component near 3400 cm^{-1} has been assigned to asymmetrical OH stretching. The later band has not been distinctively resolved in our spectra because of a drop in the

Table I. Major vibrational mode changes identified in NIR-FT spectra of the samples of normal human skin, stratum corneum, hair, and nail. Peak position, mean with 95% confidence intervals (cm^{-1})^a

	$\nu(\text{C}=\text{O})$ amide I	$\delta(\text{NH})$ and $\nu(\text{C}-\text{N})$ amide III	$\delta_{\text{as}}(\text{CH}_3)$ in proteins	$\delta(\text{CH}_2)$ scissoring in lipids and $\delta(\text{CH}_2)$, $\delta(\text{CH}_3)$ in proteins	Acyl backbone chain conformation in lipids	$\nu(\text{S}-\text{S})$ in proteins
Whole skin	1661 (1660–1663)	1271 (1270–1273)	2942 (2941–2943)	1451 (1449–1452)	1100 (1093–1107)	540 (534–546)
Stratum corneum	1649 (1647–1650)	1273 (1271–1275)	2934 (2933–2935)	1447 (1446–1448)	1125 (1124–1126) 1029 (1028–1031)	520 (515–522)
Hair	1655 (1653–1656)	1273 (1270–1275)	2928 (2926–2930)	1447 (1446–1448)	1128 (1125–1132) 1033 (1030–1053)	509 (506–511)
Nail	1649 (1647–1651)	1273 (1269–1274)	2933 (2932–2934)	1447 (1447–1448)	1127 (1125–1128) 1030 (1029–1033)	511 (509–514)

^a ν , stretching mode; ν_{s} , symmetric stretch; ν_{as} , asymmetric stretch.

detector sensitivity in this region. Thus the best estimate of total hydrogen bonded water is obtained by calculating I_{2940}/I_{3250} . This ratio was significantly lower for the whole skin (3.61 ± 1.89 ; $p < 0.001$; mean $\pm$ standard deviation) than for stratum corneum, nail, and hair (25.37 ± 8.59 ; 19.82 ± 3.02 ; 25.37 ± 8.59 , respectively), indicating the following levels of water content: whole skin > stratum corneum, nail, hair (**Fig 3f**).

Water in biologic systems is hydrogen bonded either to biomolecules (proteins) or intermolecular to other water molecules. Water molecules nonhydrogen bonded to biomolecules are ordinarily called free water, although they are hydrogen bonded to other water molecules with a tetrahedral structure (**Fig 2g**). Tetrahedron water structure is reflected by a band at $\approx 180 \text{ cm}^{-1}$, representing hydrogen bonded stretching (Walrafen, 1972; Maeda and Kitano, 1995). This motion mainly involves the oxygen atoms in the tetrahedron surroundings (Faurkov Nielsen, 1979, 1997; Brooker *et al*, 1988). This band can be visualized employing the $R(\nu)$ representation of the spectra (**Fig 3g**). In the spectra of the whole skin, stratum corneum, and nail the 180 cm^{-1} water band did not appear, indicating that the majority of water molecules are bound to other compounds and do not form tetrahedron structures. Interpretation of the hair spectra in $R(\nu)$ representation was difficult because of a low signal/noise ratio.

DISCUSSION

In this study we performed Raman spectroscopic investigations of human skin, stratum corneum, hair, and, which to determine the conformation of proteins, lipids, and water structure. We showed that it is possible to obtain highly reproducible spectra with this technique. We were particularly interested in finding a correlation between the conformation of the molecules and the macroscopic properties of human integumental tissues. Hair and nail, which represent tissues of high chemical and mechanical resistance, had a very highly ordered protein structure. The proteins were predominantly in the α -helix conformation and were heavily folded. A high degree of folding is probably responsible for the chemical resistance because only a minimal interaction is allowed between the protein and its aqueous environment. The chemical and mechanical resistance of the proteins is further reinforced by multiple disulfide links. The S–S bonds in the hair and the nail proteins are very stable (the *gauche-gauche-gauche* conformation). As has been shown for albumin and acid phosphatase isoenzymes, the amount of *gauche-gauche-gauche* S–S bonds correlates directly with the resistance of the protein to denaturation (Aoki *et al*, 1982). Similarly, the extremely high stability of snake neurotoxins in the presence of denaturing factors is mainly mediated by *gauche-gauche-gauche* S–S bonds (Tu *et al*, 1976). In the stratum corneum, the disulfide crosslinks are predominantly in the *gauche-gauche-trans* conformation. Thus, compared with the hair and the nail, not only is the total number of

crosslinks reduced but their conformation is less stable as well. The proteins in stratum corneum are arranged in α -helices but the degree of protein folding, as judged by C–H bonds, is slightly lower. In the spectra of the whole skin, which additionally contains components originating from the viable epidermis and the dermis, the structure of proteins is even more loose, allowing for the interaction between various proteins and their microenvironment. Such structure has inevitably less mechanic resistance in comparison with the hair and the nail, but allows for a better hydration of protein molecules. Protein–water interaction allows for the metabolic processes to occur and from the mechanic point of view is essential for the flexibility and elasticity of stratum corneum (Blank, 1952; Imokawa and Hattori, 1985; Jokura *et al*, 1995).

We found that in stratum corneum, nail, and the whole skin, water was present mainly in the bound form. The question of the structure of water in the skin has been the subject of numerous investigations, but the relative proportions of bound and free water have not been defined conclusively. The problem with methodologies used to date is that they assess the proportion of bound water indirectly, e.g., as the fraction of nonfreezable water in thermal analysis, or changes in relaxation times in nuclear magnetic resonance (Bulgin and Vinson, 1967; Walkley, 1972; Gniadecka, 1997). Bound water is properly defined as that forming heterogeneous structures with other molecules via hydrogen bonds (Cavotorta *et al*, 1976; Faurkov Nielsen, 1993, 1997). Water molecules that are not bound predominantly form tetrahedron clusters (Maeda and Kitano, 1995). Raman spectroscopy enables the amount of total water (OH stretching band at around 3250 cm^{-1}) and tetrahedron water (the band around 180 cm^{-1} in the $R(\nu)$ representation) to be calculated (Faurkov Nielsen, 1993, 1997; Maeda and Kitano, 1995).

The tetrahedron water specific band was not detectable in either the investigated samples. Preliminary Raman spectroscopy studies on lysozyme showed that bulk water (tetrahedron clusters) is formed with more than 10% of water in the lysozyme water mixture (Faurkov Nielsen *et al*, 1997). Bearing in mind the absence of a water free band in the investigated samples, one could speculate that over 90% of total water molecules in the whole skin, stratum corneum, and nail are in the bound form.

Differential thermal analysis studies showed that bound water presented 34% of normal stratum corneum dry weight (Bulgin and Vinson, 1967; Walkley, 1972), which means $\approx 80\%$ of the total stratum corneum water. Thus these studies imply that around 20% of stratum corneum water is free, which is close to our results.

In human skin the lipids play a major role in forming the permeability barrier. Earlier studies with X-ray diffraction and nuclear magnetic resonance spectroscopy on isolated corneocytes and lipids revealed that the lipids are firmly attached to the protein envelopes, forming a water-

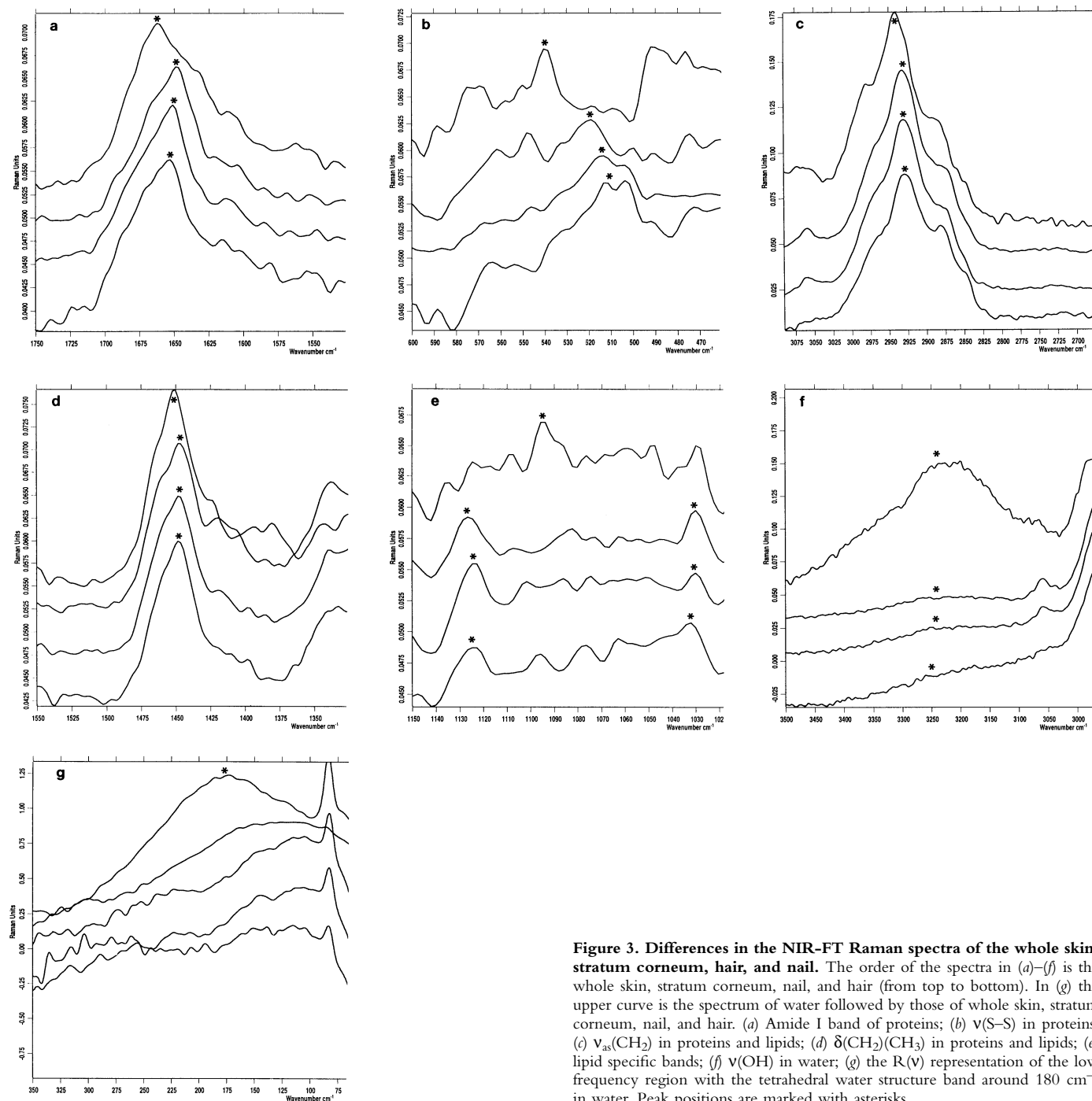


Figure 3. Differences in the NIR-FT Raman spectra of the whole skin, stratum corneum, hair, and nail. The order of the spectra in (a)–(f) is the whole skin, stratum corneum, nail, and hair (from top to bottom). In (g) the upper curve is the spectrum of water followed by those of whole skin, stratum corneum, nail, and hair. (a) Amide I band of proteins; (b) v(S-S) in proteins; (c) $\nu_{as}(\text{CH}_2)$ in proteins and lipids; (d) $\delta(\text{CH}_2)(\text{CH}_3)$ in proteins and lipids; (e) lipid specific bands; (f) v(OH) in water; (g) the R(v) representation of the low frequency region with the tetrahedral water structure band around 180 cm⁻¹ in water. Peak positions are marked with asterisks.

impermeable external sheet (White *et al*, 1988; Lazo *et al*, 1995). Surprisingly, it was found that the proteins that interact with the lipids in corneocytes were in the beta-sheet conformation (Wertz *et al*, 1989). This finding is not supported by our results, which show that in the stratum corneum the vast majority of proteins are in the α -helical conformation. A tight packing of the lipids suggests that only a small proportion of lipids in the intact tissue interacts with structural proteins; however, despite a lack of significant interactions with the proteins, the structure of lipids matches their role in the formation of the barrier. As shown here, lipids have a highly ordered lamellar crystal structure with a very low degree of fluidity. It is conceivable that such lamellar structures form hydrophobic sheets protecting against both water loss and penetration through the stratum corneum. This matches a previously postulated interaction between water and lipids in the stratum corneum (Imokawa *et al*, 1991; Imokawa, 1994), where lipid lamellae could form water holding reservoirs.

In summary, Raman spectroscopy provides a feasible method for investigating the structure of protein, lipids, and water in intact skin, hair, and nail samples. Interpretation of Raman spectra revealed that protective, mechanically resistant structures such as stratum corneum, hair, and nail, present a highly ordered protein (folded and S-S crosslinked α -helix) and lipid (lamellar crystal) molecules held together by hydrophobic interactions.

The study was supported by the Danish Cancer Society grant nr: 9610036 and Novo Nordisk Foundation, Aage Bangs Foundation and Lægeforeningens Forskningsfond, Denmark. The IFS66 and 106 NIR-FT-Raman instrument were funded by the Material Science Programme and Haldor Topsøe A/S Lyngby, Denmark. Daniel H. Christensen and Ole F. Nielsen wish to thank the Danish Natural Science Research Council for general funding during the project.

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