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Thermally stimulated current changes of irradiated skin

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Radiation effects on collagen and skin have been characterized by means of thermally stimulated current (TSC) spectroscopy. X-rays, β-rays and UV radiation have similar effects on the molecular mobility of dermal collagen and skin. They induce a decrease of intra and intermolecular mobility. The restriction of molecular movement can be explained by an increase of collagen cross-links.

1. Introduction

It is well established that excessive exposure of skin to sunlight leads to severe damage to the underlying connective tissue i.e. dermis. These changes are attributed to ultraviolet radiation (UV) [1]. Moreover, skin can sometimes, in particular for therapeutic purposes, be exposed to more energetic radiations i.e. X, γ or β rays, which could cause profound alterations of the dermal proteins. Nevertheless, there are few data for measurements of the effects induced by these radiations, and reports deal mainly with the macroscopic changes of skin [2–4]. On the other hand, several histochemical and biochemical studies on UV damaged skin, have shown accumulation of elastic materials and glycosaminoglycans [5–8]. However, there is some biochemical evidence for modifications of collagen [9, 10]. Since collagen is the major component of normal skin (70–80% of the dry weight), it is important to identify the mechanisms responsible for radiation-induced changes in this protein. Thus, in the present work we focus on the study of the molecular movement changes induced by radiations, in dermal collagen and skin, in order to estimate the damage induced at different levels of collagen organization, i.e. peptid chains, molecules and fibrils. This investigation was achieved by means of dielectric spectroscopy; thermally stimulated currents.

2. Materials and method

2.1. Animals and irradiation schedules

Ionizing radiations. Irradiations were carried out on the flank skin of 3–4-month-old female large white pigs. Fields of 4 cm × 2 cm were irradiated by a single dose with X-rays ($E = 0.25$ keV) and with β emitters of high and low energies, ^{90}Sr ($E = 2.27$ MeV) and ^{170}Tm ($E = 0.76$ MeV), respectively. Skin samples were removed by a full thickness incision from control and irradiated animals, then they were frozen at $-20^{\circ}C$.

Non-ionizing radiations. Irradiations were carried out on the flank skin of 12-month-old male rats. The animals were irradiated five times a week for 10 months. The daily doses of UVB and UVA were 1 J/cm^2 and 10 J/cm^2, respectively. The ultraviolet source was "Biotronic" (from Vilbert Lourmat). Strips of flank skin were removed from control and irradiated groups and frozen at $-20^{\circ}C$ prior to collagen extraction. Collagen was isolated from other skin proteins by acid extraction, pepsin digestion and salt precipitation (Fig. 1), then the extracts were freeze dried.

2.2. Thermally stimulated current (TSC) spectroscopy

With TSC spectroscopy, the sample is subjected to a static electric field for 2 min at a temperature T_p allowing the mobile units to orientate. This out-of-equilibrium configuration is then "frozen-in" by a rapid quench to a temperature T_0 ($T_0 \ll T_p$) and the electric stress is removed. The return to equilibrium state of the sample is induced by a controlled increase of temperature, and the depolarization current is recorded versus temperature, giving the TSC spectrum.

For TSC experiments, a static voltage of 300 V was applied at room temperature and the temperature of the sample was decreased to liquid nitrogen temperature. Before the TSC experiments, skin and collagen samples were kept at a temperature of 110 °C under

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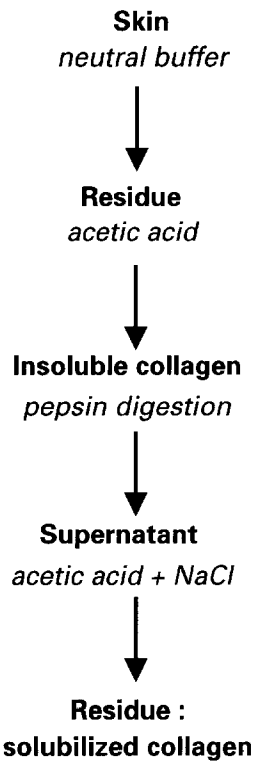

Figure 1 Extraction of dermal collagen.

1.32×10^{-4} Pa vacuum for 12 h: this procedure is reported to completely remove the water [11].

For each treatment, several samples were investigated in order to verify the homogeneity of the response. All TSC spectra have been normalized to the external electrical field and to the surface of the sample. Thus on TSC spectra we have reported the dynamic conductivity: $\sigma = I\,e/s\,V$, where I is the depolarization current, V is the static voltage, e and s are, respectively, the thickness and the area of the sample.

3. Results

3.1. TSC relaxations of collagen and skin

3.1.1. Collagen

The TSC spectrum of collagen shows three dielectric relaxation maxima, in the temperature range -180–$60\,^{\circ}C$ (open circles on Fig. 2). These occur around -150, $-50\,^{\circ}C$ and room temperature. They have been labelled γ, β and α peaks, respectively.

It should be stressed that the magnitude of the low temperature modes (γ and β) is 100 times lower than that of the room temperature mode, hence the movements involved in the latter relaxation are delocalized while those involved in the former modes are localized.

On the basis of studies undertaken on the influence of water on collagen TSC relaxations in collagen [12] and of mechanical and dielectric analysis in the frequency range [11, 13], the γ and β peaks have been attributed to local motion of apolar and polar regions in collagen, respectively.

On the other hand, the assignment of the predominant process (α) is based on data obtained by Lamure *et al.* [14] on different connective tissues. The authors have attributed this peak to intermolecular movements along the triple helices of collagen. It is important to note that analysis of the kinetic mode showed that these latter movements are ruled by free volume in the collagenous matrix.

Overall, TSC experiments reflect the structural hierarchy of collagen and allow one to distinguish between intramolecular (γ and β) and intermolecular (α) movements.

3.1.2. Skin

Results obtained on skin samples bear a general resemblance to those of collagen. As shown on Fig. 2 (close circles), collagen dielectric peaks are also observed on the TSC spectrum of skin. Despite this similarity, some differences in the relaxations position and intensity can be noted.

First, the α peak magnitude of skin is approximately 10 times as intense as that of collagen ($\sigma \approx 1.5 \times 10^{-11}$ S/m for skin and $\sigma \approx 10^{-12}$ S/m for collagen). A second discrepancy comes from comparison of the β peak position in skin and collagen: for collagen this relaxation occurs at around $-50\,^{\circ}C$ whereas for skin this mode is observed at around $-80\,^{\circ}C$. An explanation for the peak shift may be related to the presence of non-collagenous proteins. These proteins include

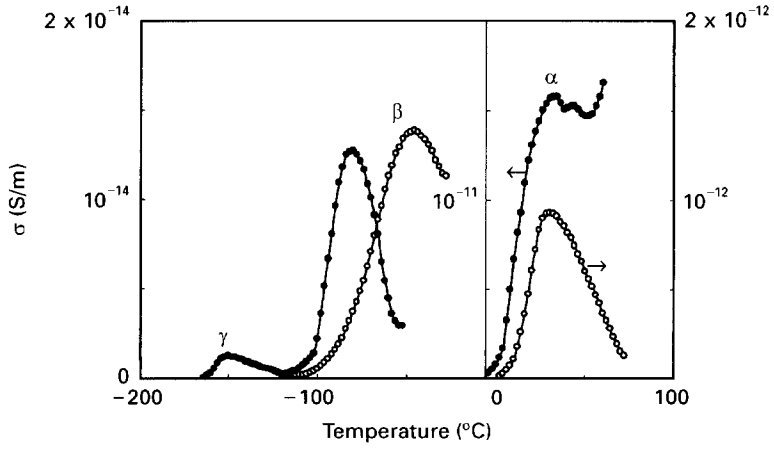

Figure 2 TSC spectra of collagen (-⊖-) and skin (-●-).

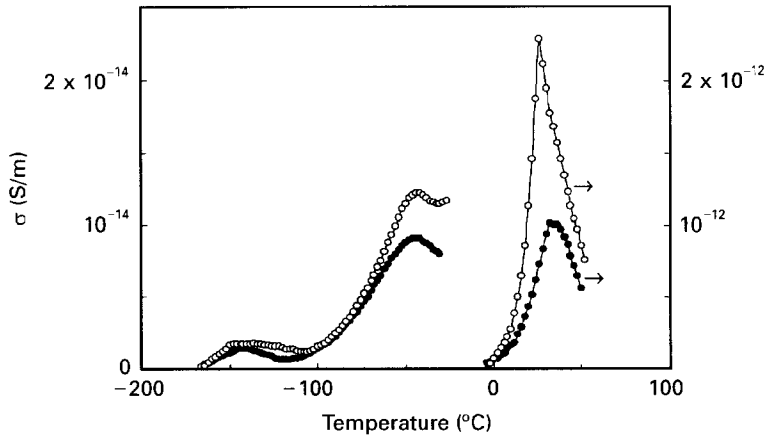

Figure 3 Evolution of collagen TSC spectra upon UV exposure: -⊖- control; -●- UV irradiated.

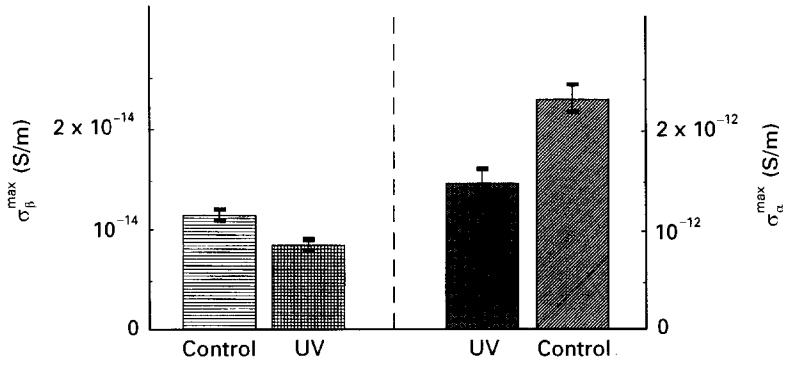

Figure 4 Evolution of the magnitude of the β and α modes under UV radiation.

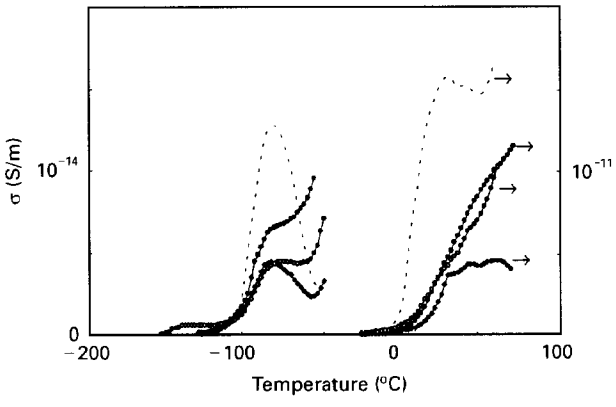

Figure 5 TSC spectra of control (- - -) and irradiated skin samples: Sr -●-; T_m -◆-; Rx -⊖-.

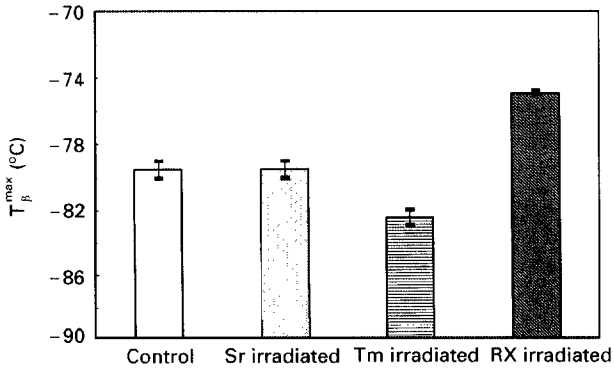

Figure 6 Kinetics of the β mode upon ionizing radiations.

hydrophilic glycosaminoglycans which can have a plasticizing action on collagen, in lowering the temperature of the β peak. In addition to the "plasticization" of polar group motion, non-collagenous proteins also give rise to an amplification of the α peak which is a direct consequence of the increase of free volume between collagen molecules in skin.

3.2. UV effects on collagen

The α, β and γ relaxation modes observed on the TSC spectrum of collagen extracted from UV irradiated skin are located in the same temperature ranges as the control samples (closed circles on Fig. 3).

There are, however, significant differences. The most striking effect of UV irradiation on the dielectric response of collagen concerns the magnitude of the modes. Upon irradiation, the TSC data show clearly that the β peak intensity is depressed; this discrepancy becomes even greater at higher temperatures (Fig. 3). In fact, the magnitude of the α process is decreased by approximately half. In contrast, the relaxation intensity of the γ peak remains constant after irradiation.

In order to verify the reproducibility of these evolutions several scans were carried out on four different animals (for each group). Then a statistical analysis, using the Student *t*-test was performed to determine data significance. The mean values and standard errors derived from the analysis are reported in Fig. 4. As shown in Fig. 4, the average curves exhibit highly

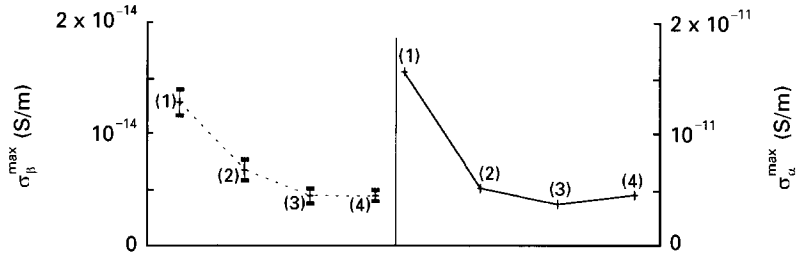

Figure 7 Evolution of the magnitude of the β and α modes under ionizing radiations: (1) control; (2) Sr irradiated; (3) T_m irradiated; (4) RX irradiated.

significant magnitude decreases of both β and α peaks, i.e. σ_β^{max} and σ_α^{max}, upon irradiation (errors: $p < 0.01$ and $p < 0.001$, respectively).

3.3. Effects of X and β rays on skin

The TSC spectra obtained for control and irradiated skin are reported in Fig. 5. It is important to note that the γ peak is not always well resolved, thus it has not been included in the discussion. Also, for irradiated skin the higher temperature mode α appears only as a shoulder making its kinetic analysis quite difficult. Conversely, the β peak is always well resolved and a statistical analysis of the temperature peak (T_β^{max}) showed that:

(a) upon exposure to β-rays from an Sr source, the position of the peak remains constant (Fig. 6);
(b) In contrast, a significant shift is observed for $^{170}T_m$ and RX irradiations. For T_m irradiated samples the peak shifts downwards towards $-83\,^\circ C$, whereas for RX irradiation the temperature of the β peak increases to $-75\,^\circ C$ (Fig. 6).

As for the changes induced by UV radiation, the most striking evolution, observed for ionizing radiations, is a restriction of the magnitude of TSC peaks (α and β) of irradiated samples compared with unirradiated samples. Statistical analysis of the magnitude of α and β mode values revealed that σ_β^{max} was reduced by a factor two while σ_α^{max} was reduced by a factor three (Fig. 7).

4. Discussion

Due to the preliminary nature of the results presented in this work, we cannot completely elucidate the specific mechanisms underlying these radiation-induced effects on the molecular organization of skin. Instead, in this discussion we will compare the dielectric relaxation behaviour of skin and collagen after exposure to ionizing and non-ionizing radiations. Before proceeding with this comparison, it would be useful to examine similarities in skin and collagen responses. The TSC spectra of both collagen and skin show three relaxation processes (α, β and γ) having quite similar characteristics (position and intensity). However, the main difference between the TSC responses can be directly attributed to the action of non-collagenous proteins. Since, the β peak involves motion of polar regions, the presence of hydrophilic protein like glycosaminoglycans, can strongly influence the peak position. Moreover, the observation of a significant increase of the α peak intensity can also be associated with the presence of non-collagenous proteins which increase the free volume between collagen molecules.

Despite, these differences, TSC relaxation processes of skin have probably the same origin as those of collagen. This correlation is further substantiated by similarities in collagen and skin behaviour upon irradiation.

Effects of non-ionizing radiations (UV) investigated on purified collagen reflect a significant difference of the magnitude of both β and α peaks. It is logical to think that the reduction of intra and intermolecular mobilities causes stiffening of the collagenous matrix induced by an increase of cross-links. This hypothesis is supported by recent works [15–18], which have shown that exposure to UV radiation leads to cross-linking reactions in collagen.

On the other hand, study of the effects of ionizing radiations (β and X-rays) has been achieved directly on skin samples. The TSC spectra also show a significant decrease in the α and β peaks. As for UV effects, the mobilities restriction induced by ionizing radiations can be explained by the cross-linking process in proteins, especially in collagen. This explanation is also in agreement with data obtained with γ irradiation [19].

Otherwise the analysis of the β peak position reflects the evolution of the kinetics of intramolecular mobility in a selective manner according to the nature and the energy of the irradiating particles: $^{170}T_m$ irradiation softens the intramolecular mobility, while X-ray irradiations stiffens the collagen polar sequences.

5. Conclusions

TSC spectroscopy has been applied to study radiation effects on skin. For the characterization of non-ionizing radiations, the TSC spectrum of collagen has been used as reference. Under irradiation, collagen maintains its integrity, but its molecular mobility is decreased. This evolution has been ascribed to an increase of cross-links. The influence of ionizing radiations has been directly controlled on skin. For all irradiations, restriction of molecular mobility, analogous with that induced by UV irradiations, has been exhibited.

References

[1] L. H. KLIGMAN, F. J. AKIN and A. M. KLIGMAN, *J. Invest. Dermatol.* **78** (1982) 181.
[2] D. M. PEEL, J. W. HOPEWELL, J. WELLS and M. W. CHARLES, *Radiat. Res.* **99** (1984) 372.

[3] D. M. PEEL, J. W. HOPEWELL, J. WELLS and M. W. CHARLES, *ibid.* **101** (1985) 491.
[4] M. R. BAKER, D. BADER and J. W. HOPEWELL, *Brit. J. Radiol.* **62** (1989) 830.
[5] K. J. JOHNSTON, A. I. OIKARINEN, N. J. LOWE, J. G. CLARK and J. UITTO, *J. Invest. Dermatol.* **82** (1984) 587.
[6] E. SCHWARTZ, *ibid.* **91** (1988) 158.
[7] L. H. KLIGMAN, *J. Amer. Acad. Dermatol.* **21** (1989) 623.
[8] R. CHARTTERJEE, M. J. BENZINGER, J. L. RITTER and D. L. BISSET, *Photochem. Photobiol.* **51** (1990) 91.
[9] S. R. PLASTOW, J. A. HARRISON and A. R. YOUNG, *J. Invest. Dermatol.* **91** (1988) 590.
[10] L. H. KLIGMAN, M. GEBRE, A. ALPER and N. A. KEFALIDES, *ibid.* **93** (1989) 210.
[11] S. NOMURA, A. HILTNER, J. B. LANDO, and E. BEAR, *Biopolymers* **16** (1977) 231.
[12] S. MEZGHANI, A. LAMURE and C. LACABANNE, *J. Polym. Sci. Polym. Phys. Edn* (in press).
[13] E. P. CHANG and J. C. W. CHIEN, *J. Polym. Sci. Polym. Phys. Edn* **11** (1973) 737.
[14] A. LAMURE, N. HITMI, C. LACABANNE, M. F. HARMAND, and D. HERBAGE, *IEEE Trans. Electric. Insulation* **EI-21** (1986) 443.
[15] L. H. KLIGMAN and M. GERBE, *Photochem. and Photobiol.* **54** (1991) 233.
[16] H. NIEDERMÜLLER and R. D. PETERSEN, *Arch. Gerontol. Geriatr.* **suppl. 3** (1992), 277
[17] M. DALLE CARBONARE and M. A. PATHAK, *J. Photochem. Photobiol. B: Biol.* **14** (1992) 105.
[18] M. J. FRYER, *Photochem. and Photobiol.* **58** (1993) 304.
[19] F. JAROSZYK and E. MARZEC, *J. Mater. Sci.* **29** (1994) 5353.

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