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Photochemical transformations in collagen in the presence of melanin

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Abstract

The influence of UV radiation (254 nm) on collagen from rat tail tendon in the absence and presence of melanin was investigated. It was found that the relative viscosity and fluorescence of collagen decreased, whereas the absorption/scattering of collagen solution increased during irradiation of the sample. Such changes in the physical properties indicate that photodegradation, phototransformation and changes in the conformational state of collagen take place during UV irradiation. The changes in the above properties in the presence of melanin suggest that it makes collagen more resistant to the action of UV radiation.

Keywords: Collagen; Rat tail tendon; UV radiation; Melanin

1. Introduction

Collagen is the major compound of human skin and one of the most abundant proteins in the human body [1,2]. Solar UV radiation plays a major role in the pathogenesis of sunburn reaction, skin cancer and solar photoaging of human skin [3–5]. The solar radiation is a complex spectrum of radiation including UVC (220–290 nm), UVB (290–320 nm) and UVA (320–400 nm), as well as visible light and infrared radiation. Many studies demonstrated physicochemical changes of collagen induced by UV radiation. It has been shown that the solution of collagen, after irradiation, loses the ability to form natural fibrils [6].

The fluorescence observed after UV irradiation is due to the presence of phenylalanine and tyrosine in this protein [7–9]. Photocrosslinking [10–12] and photodegradation [9,13] of collagen may also occur during exposure to UV radiation. All of the above reactions may be attributed to direct absorption by tyrosine/phenylalanine or to peptide bonds. Because of the negligible absorption of all of these species above 300 nm, these processes would probably not play a significant role in in vivo collagen photolysis by solar radiation. Recently the solar UV radiation has increased at the earth's surface [14]. A change of the radiation environment could have serious consequence on the biosphere [15,16], because the type of photochemical process and its efficiency depend on the atmosphere [17], pH of collagen solution [18], type of collagen and its age [19,20], light wavelength [21,22] and the presence of other substances [21,23–27]. In living organisms photoprotection agent against UV damage of collagen skin is melanin. Clinical, epidemiological and experimental evidence indicates a protective role for constitutive melanin with respect to solar UV [28–31]. The melanin protects the organisms against solar radiation by absorption and scattering of harmful UV [32] and by binding a wide variety of compounds [33]. It acts as radical scavengers in photoprotection [34,35] and as an electron transport conduit [36–38].

The aim of this study was to determine the ability of melanin to protect collagen in vitro against changes induced by UV irradiation (254 nm).

2. Materials and methods

Collagen was obtained in our laboratory from tail tendons of young albino rats. We used the same method as previously employed [26,27]. After washing in distilled water these tendons were dissolved in 0.04 M acetic acid solution. Melanin was obtained from Sigma.

The samples in the form of the solutions of pure collagen and collagen containing melanin (1 mg of melanin in 1 ml of collagen solution) were irradiated under air at room temperature using a mercury lamp, Philips TUV-30, which emits light of mainly 254 nm wavelength. The intensity of radiation was 0.263 J/cm^2 min. Dose of incident radiation during 1 h exposition was 16 J/cm^2. The intensity of the incident light was measured using an IL 1400A Radiometer (International Light, USA). Irradiation experiments were carried

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out in a quartz cuvette at a distance of 3 cm from the light source.

The UV-VIS absorption spectra of the collagen solution, before and immediately after UV irradiation were recorded with a Shimadzu spectrophotometer (Model UV-1601PC). Data collection and plotting were accomplished by the UVPC program and the computer data station supplied by the manufacturer.

The intrinsic fluorescence studies were performed on a Perkin–Elmer spectrofluorimeter equipped with a differential corrected spectral unit. Spectra were gathered using the Perkin–Elmer Data Manager on Perkin Elmer Computer Spectroscopy Software (PECSS) program to obtain the differential excitation and emission scan.

The relative viscosity of collagen solution was measured at 20°C using a quartz Ubbelohde viscometer.

3. Results and discussion

The absorption spectra of a solution of acid soluble type 1 rat tail tendon collagen in acetic acid reveals a highly scattering solution with absorption in the 250–280 nm region. Under UV irradiation, the UV-VIS spectra which characterise the collagen solution changed distinctly (Fig. 1, curves 1–6). Irradiation of collagen solution with 254 nm wavelength leads to a minor increase in overall absorption, most notably between 240–300 nm. This may be mainly an effect of increasing of turbidity in irradiated solution, because the UV irradiation causes the changes in conformation of collagen molecule (helix-coil transition). After 1 and 2 h UV irradiation the maximum of absorption/scattering is almost the same. This fact shows that after 1 h irradiation collagen molecules change totally their conformational state.

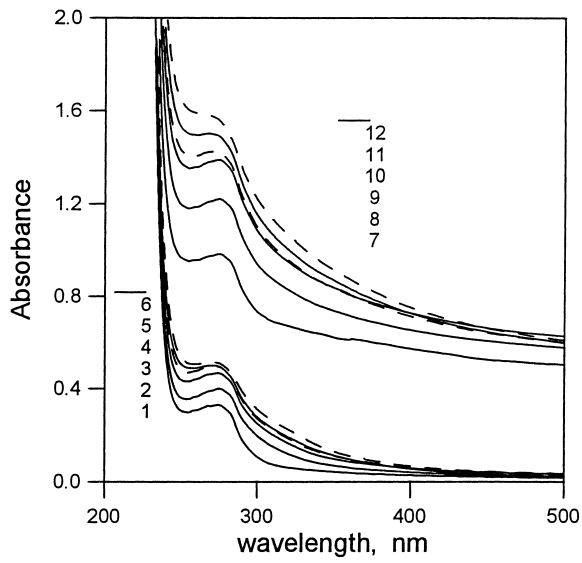

Fig. 1. UV-VIS spectra of collagen (curves 1–6) and collagen containing melanin (curves 7–12) before and after UV radiation for: 1,7 – 0 min; 2,8 – 5 min; 3,9 – 15 mm; 4,10 – 30 min; 5,11 – 1 h; 6,12 – 2 h.

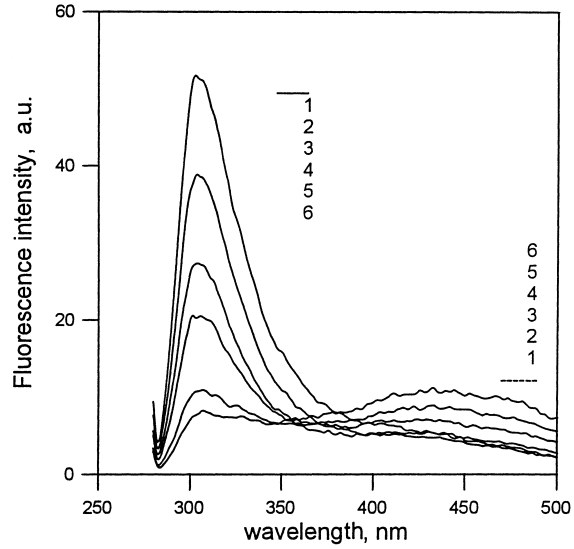

Fig. 2. Effect of UV radiation on fluorescence spectra of collagen (excitation at 275 nm): 1 – 0 mm; 2 – 30 mm; 3 – 1 h; 4 – 2 h; 5 – 4 h; 6 – 8 h.

In the presence of melanin, the change in absorption/scattering occurs slowly (Fig. 1, curves 7–12). The difference between absorption/scattering after 1 and 2 h irradiation is bigger than in pure collagen. This suggests that melanin increases the photochemical stability of this protein and hinders the processes which lead to the increase of absorption/scattering, i.e. photodegradation (with scission of bonds in the main chains) or phototransformation (with changes in the conformation of collagen molecules).

The maximum of absorption/scattering of collagen solution is observed at 275 nm wavelength. Excitation at 275 nm affords UV fluorescence. The corrected fluorescence spectrum shows maximum at 305 nm (Fig. 2). The peak at 305 nm may be attributable to tyrosine which is present in polypeptide chains of collagen [39]. The fluorescence of collagen is predominantly due to tyrosine residues, however, all the aromatic residues are being excited to the same extent at 270–290 nm wavelength. We observed UV-induced fluorescence fading at 305 nm wavelength in collagen. The kinetics of fluorescence fading depends on the time of irradiation, on the irradiation wavelength and the previous history of the sample [40]. As tyrosine fluorescence decreases, another broad, weak band, attributable to dityrosine [41], emerges in this region with emission maximum at 400–500 nm wavelength. A new band around 420–500 nm wavelength appears after 1 h irradiation (Fig. 2, curve 3). The control samples did not show significant emission in this region (Fig. 2, curve 1). It is known that tyrosine is converted to the tyrosyl radical by various oxidising agents and that two tyrosyl radicals react with each other, accompanied by the formation of dityrosine [42]. Formation of dityrosine and other tyrosine photoproducts are the consequence of the

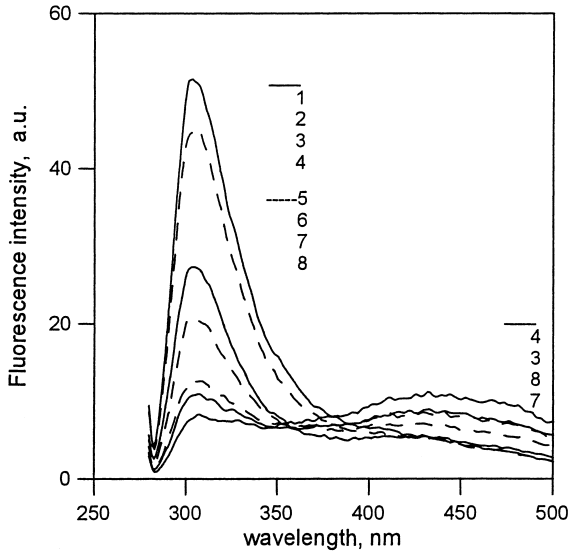

Fig. 3. Effect of UV radiation on fluorescence spectra of collagen (———) and collagen containing melanin (- - -) (excitation at 275 nm): 1,5 – 0 min, 2,6 – 1 h; 3,7 – 4 h; 4,8 – 8 h.

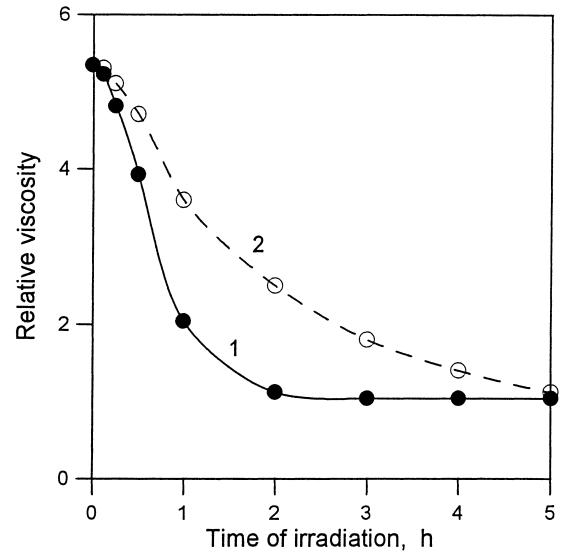

Fig. 4. Changes in η_{rel} of collagen (curve 1) and collagen containing melanin (curve 2) during UV radiation.

photoionisation and radical generation process, which predominate with tyrosine photochemistry. The qualitative and quantitative nature of photochemical fluorescence fading is highly sensitive to collagen conformation.

The fading fluorescence of collagen under UV radiation is inhibited in the presence of melanin (Fig. 3). In the presence of melanin the change in fluorescence intensity occurs slowly. Comparison of the fluorescence at 305 nm after 8 h UV irradiation in both samples (collagen and collagen mixtures with melanin) leads to the conclusion that greater changes of fluorescence are in pure collagen sample than in the sample containing melanin (Table 1). Similarly, the new band intensity around 420–500 nm is greater in the sample without melanin. In the presence of melanin this band appears after 4 h irradiation. This suggests that melanin increases the photochemical stability of collagen type 1 and hinders phototransformation. This is confirmed by viscosity measurements.

On UV irradiation, the relative viscosity (η_{rel}) of collagen decreases rapidly, and then remains at a stable low level without any changes with further irradiation (Fig. 4, curve 1). In the presence of melanin, the change in η_{rel} occurs slowly (Fig. 4, curve 2) and, for the irradiation time used, does not reach a stable state. This confirms that melanin increases the photochemical stability of collagen.

The above results i.e. differences in intensity of tyrosine excitation and in changes of fluorescence intensity after UV irradiation suggest that the photochemical reaction in collagen are restrained by melanin.

Melanin absorbs light and dissipates most of its energy into heat. However, small fraction of the excitation energy can dissipate through chemical reactions. It is well established that light induces redox equivalents on melanin polymer and aerobic photoirradiation of isolated melanin pigments leads to consumption of oxygen with formation of superoxide anion intermediate and stoichiometric release of hydrogen peroxide. Because melanin absorbs more light as the wavelength decreases, it is expected to be more photoprotective at shorter wavelengths, provided protection is due to the absorption of photon, i.e. due shielding. If protection is due to scavenging of reactive oxygen species, then it would be difficult to predict the wavelength dependence

Table 1
Changes of fluorescence intensities $\Delta F = F_0 – F_t$ (F_0, fluorescence intensity before irradiation, and F_t, after irradiation) of collagen and collagen containing melanin

Time of irradiation (h)	Dose of incident radiation (J/cm^2)	ΔF at 305 nm		ΔF at 400–500 nm	
		collagen	collagen + melanin	collagen	collagen + melanin
0	0	0	0	0	0
0.5	8	−14	−4	0	0
1	16	−24	−8	0.5	0
2	32	−30	−18	1.5	0
4	64	−41	−31	5.5	1
8	128	−44.5	38.5	7	2

because so little is known about the nature and yield of such species at different wavelengths.

The molecular mechanism of photoprotecing of melanin is not fully understood. The ability of melanin to scavenge free radicals is likely to depend on their physico-chemical properties – their lifetime, one electron reduction potential and net charge. Melanin can also interact efficiently with tryptophan, tyrosine and nitrogen dioxide, which are oxidizing species.

Melanin protection against photochemical changes in collagen appears to take place primarily via optical absorption and scattering.

Melanin may provide protection against photochemical destruction of fluorescent chromophores, primarily by a mechanism involving absorption and scattering of impinging radiation away from the target chromophore molecules.

4. Conclusion

Melanin acts as a photostabilizer of collagen. This pigment decreases the structural changes of collagen caused by irradiation at 254 nm mainly by absorption and scattering of incidence radiation.

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