ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 120 (1999) 207–210

Journal of Photochemistry and Photobiology A:Chemistry

Changes induced by ultraviolet light in fluorescence of collagen in the presence of β-carotene

Alina Sionkowska*, Alina Kamińska

Faculty of Chemistry, N. Copernicus University, 87-100 Toruń, Poland

Received 11 September 1998; accepted 19 October 1998

Abstract

The influence of UV radiation (253.7 nm) on collagen fluorescence in the absence, and presence, of β-carotene was investigated. It was found that UV radiation of 253.7 nm causes irreversible destruction of tyrosyl and phenylalanyl residues. The fluorescence of collagen (excitation at 275 nm, emission at 305 nm) decreased rapidly during irradiation and a new fluorescence large band at 400–500 nm formed under UV radiation. Smaller changes in the fluorescence of collagen in the presence of β-carotene suggest that it makes collagen less sensitive to the action of UV radiation. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Collagen; Rat tail tendon; UV radiation; Fluorescence; β-Carotene

1. Introduction

Collagen is one of the most important proteins in living organisms. It is found in the connective tissue and skin. Intra- and intermolecular hydrogen bonds in the polypeptide chains of collagen lead to formation of a triple helix [1]. Collagen contains aromatic amino acids (phenylalanine and tyrosine), capable of absorbing in the far-ultraviolet (250–280 nm) range. Many studies demonstrated physico-chemical 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 [2]. Additionally, the fluorescence observed after UV irradiation is due to the presence of phenylalanine and tyrosine in this protein [3–5]. Photocross-linking [6–8] and photodegradation [5,9] 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 wavelengths. However, the type of photochemical process and its efficiency depend on the atmosphere [10], pH of the collagen solution [11], type of collagen and its age [12,13], wavelength of light [14,15] and the presence of other substances in the protein [14,16–18].

In our previous investigation, we found that radiation with a wavelength of 253.7 nm caused changes in the relative viscosity, levorotation and polydispersity of collagen type 1 from rat tail tendon (RTT) [19]. Such changes indicated that photodegradation and phototransformation of collagen takes places during UV irradiation. In the presence of β-carotene, these changes were smaller than in pure collagen.

The aim of this study was to determine the fluorescence changes in collagen on UV irradiation (253.7 nm) and the effect of β-carotene on the fluorescence of this protein.

2. Materials and methods

Collagen was obtained in our laboratory from tail tendons of young albino rats [20]. We used the same method as previously employed [14]. After washing in distilled water these tendons were dissolved in 0.04 M acetic acid solution. β-Carotene was obtained from Sigma.

The samples in the form of solutions of pure collagen and collagen containing 0.01% of β-carotene were irradiated at atmospheric pressure and room temperature using a mercury lamp, Philips TUV-30, which emits light of mainly 253.7 nm wavelength. The intensity of radiation was 0.263 J/cm^2 min. The dose of incident radiation during 1 h exposition is 16 J cm^{-2}. The intensity of the incident light was measured using an IL 1400A radiometer (International Light, USA). Irradiation experiments were carried out in a quartz cuvette at a distance of 3 cm from the light source.

*Corresponding author.

1010-6030/99/$ – see front matter © 1999 Elsevier Science S.A. All rights reserved.
PII: S1010-6030(98)00427-4

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 manufacture.

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.

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 aromatic absorption in the 250–280 nm region. Under UV-irradiation, the UV-VIS spectra which characterize the collagen solution changed distinctly (Fig. 1). Photolysis of collagen solutions with short wavelength UV leads to a minor increase in overall absorption, most notably between 240–300 nm. This may be largely an effect of increasing of turbidity in the irradiated solution, which is noticeable to the eye. 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 a maximum at 305 nm (Fig. 2). The peak at 305 nm may be attributed to tyrosine which is present in polypeptide chains of collagen [21]. The fluorescence of collagen is predominantly due to tyrosine residues; however, all the aromatic residues are being excited in the same range at 270–290 nm. We observed

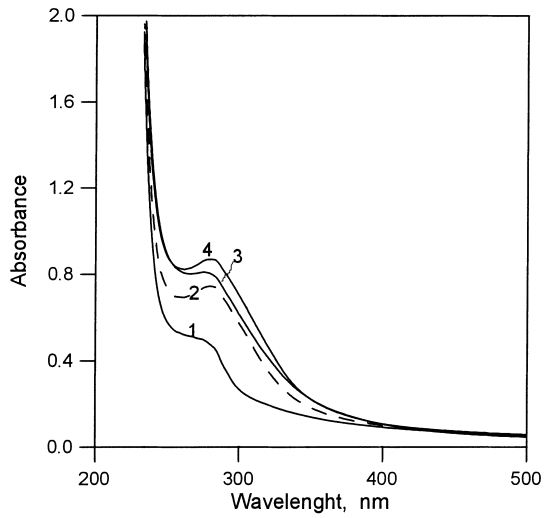


Fig. 1. UV-VIS spectra of collagen in acetic acid solution before, and after, UV radiation for: (1) 0, (2) 5, (3) 30, and (4) 2 h.

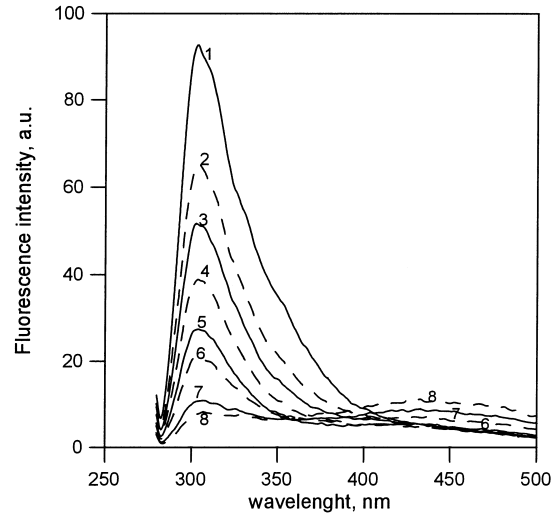


Fig. 2. Effect of UV radiation on fluorescence spectra of acid soluble collagen (excitation at 275 nm): (1) 0, (2) 5, (3) 10, and (4) 30 min, (5) 1, (6) 2, (7) 4, and (8) 8 h.

UV-induced fluorescence fading at 305 nm in collagen. The kinetics of fluorescence fading depends on the time of irradiation, the irradiation wavelength and the previous history of the sample [22]. As tyrosine fluorescence decreases, another broad, weak band, attributed to dityrosine [23], emerges in this region with emission maximum at 400–500 nm. A new band, around 420–500 nm, appears after 1 h of irradiation (Fig. 2, curve 5). 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 oxidizing agents and that two tyrosyl radicals react with each other, accompanied by the formation of dityrosine [24]. Formation of dityrosine and other tyrosine photoproducts are a consequence of the photoionization and radical generation process, which predominate in tyrosine photochemistry.

Formation of 3,4-dihydroxyphenylalanine in UV-irradiated collagen type 1 was due to direct photolysis of tyrosine under UV radiation below 300 nm light [25,26].

The qualitative and quantitative nature of photochemical fluorescence fading is highly dependent of collagen conformation. Changes in conformation induced by photochemical damage may be readily detected as changing in fading kinetics under conditions where changes in absorption, amino acid composition and electrophoresis properties are hardly noticeable.

The fading fluorescence of collagen in the presence of β-carotene under UV radiation is shown in Fig. 3. In the presence of β-carotene the changes of fluorescence intensity occur slowly. Comparison of the fluorescence at 305 nm after 8 h UV irradiation in both kinds of samples (collagen, and collagen mixtures with β-carotene) gives greater change of fluorescence in pure collagen sample than in the presence of β-carotene (Table 1). Similarly, the new band intensity around 420–500 nm is greater in the sample without

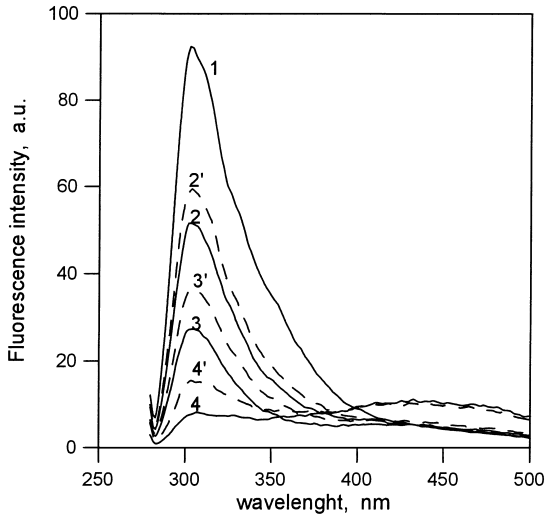


Fig. 3. Effect of UV radiation on fluorescence spectra of acid soluble collagen (———) and collagen in the presence of β-carotene (- - -) (excitation at 275 nm): (1) 0, (2, 2′) 10 min, (3, 3′) 1, (4, 4′) 8 h.

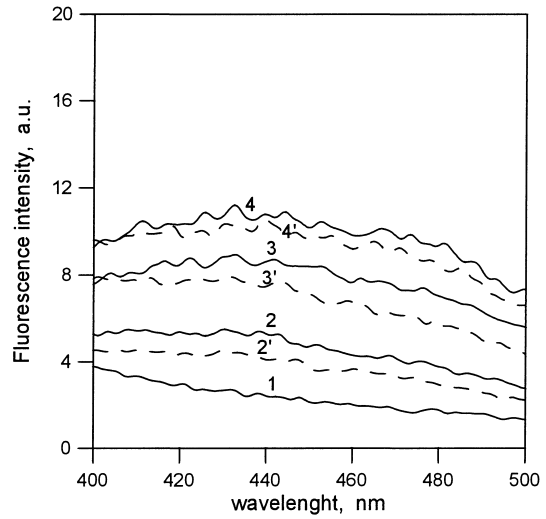


Fig. 4. Changes of fluorescence intensities at 400–500 nm after irradiation of collagen (———) and collagen in the presence of β-carotene (- - -) (excitation at 275 nm): (1) 0 min, (2,2′) 1, (3,3′) 4, (4,4′) 8 h UV.

β-carotene (Fig. 4). This suggests that β-carotene increases the photochemical stability of collagen type 1 and hinders phototransformation (with changes in the conformation of collagen molecules). This is confirmed by our previous study [19].

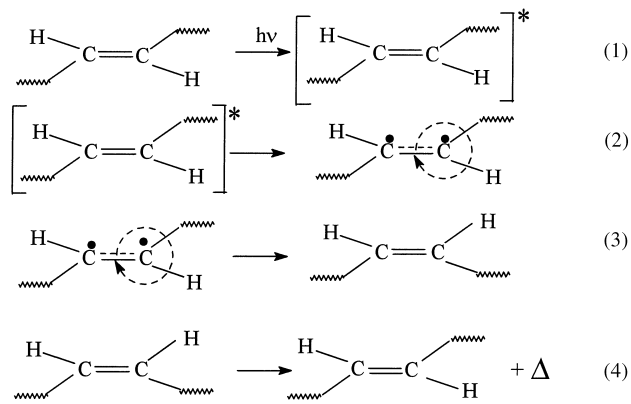
We propose the following mechanism (Scheme 1) of β-carotene inhibited phototransformation: carotene absorbs a photon and changes to an excited state (Eqs. (1 and 2)); the energy may be consumed by *cis–trans* isomerization of the β-carotene molecule (Eqs. (3 and 4)). In this manner, the possibility of photochemical transformation in collagen decreases.

Additionally, β-carotene (CAR) is an efficient quencher of oxygen molecules in the singlet state (Scheme 2), which may form under UV irradiation [27].

Oxygen in the singlet state may initiate degradation and oxidation of the aromatic amino acids like tyrosine.

The active oxygen as H_2O_2 and O_2^- can be formed from an irradiated solution of proteins [28]. These active oxygen species are converted to $HO^{\cdot}$ and $HOO^{\cdot}$, which can attack biomolecules easily. β-Carotene is an efficient quencher of these species.

The above results, namely the differences in intensity of tyrosine excitation and in changes of fluorescence intensity



Scheme 1.

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 in the presence of β-carotene

Time of irradiation (min)	Dose of incident radiation (J/cm^2)	ΔF at 305 nm		ΔF at 400–500 nm	
		collagen	collagen+β-carotene	collagen	collagen+β-carotene
0	0	0	0	0	0
5	1.33	−30	−22	0	0
10	2.66	−43	−38	0	0
30	8	−58	−54	0	0
60	16	−69	−59	1.3	0.6
120	32	−74	−66	3.8	3
240	64	−84	−76	4.5	3.6
480	128	−88	−80	7	6.5

$$^3O_2 + h\nu \rightarrow {}^1O_2$$
$$^1O_2 + CAR \rightarrow {}^3CAR + {}^3O_2$$
$$^3CAR \rightarrow {}^{\bullet}CAR^{\bullet}$$
$${}^{\bullet}CAR^{\bullet} + {}^3O_2 \rightarrow {}^{\bullet}CAROO^{\bullet}$$

Scheme 2.

after UV irradiation suggest that the photochemical reactions in collagen are restrained by β-carotene.

4. Conclusions

Our studies suggest that UV-induced changes of fluorescence may be a much more sensitive indicator of some changes of collagen structure connected with tyrosine and phenylalanine than other analytical methods. We compared photochemical stability of collagen, and collagen in the presence of β-carotene using the fluorescence method. β-Carotene acts as a photostabilizer of collagen in our conditions. This dye decreases changes in tyrosine and phenylalanine, which are responsible for collagen fluorescence.

References

[1] R. Van der Rest, M. Garrone, FASEB J. 5 (1991) 2814–2823.
[2] E. Fujimori, Biopolym. 3 (1965) 115.
[3] E. Fujimori, Biochem. 5 (1966) 1034.
[4] D.V. Crabtree, E. Fujimori, Biopolym. 19 (1980) 1081.
[5] E. Fujimori, Eur. J. Biochem. 152 (1985) 299–306.
[6] Y. Kano, Y. Sakano, E. Fujimoto, J. Biochem. 102 (1987) 839.
[7] E. Fujimori, FEBS Lett. 253 (1988) 98.
[8] A.J. Gasan, Biofizika 33 (1988) 772.
[9] S.F. Curran, M.A. Amoruso, D.B. Goldstein, FEBS Lett. 76 (1981) 155.
[10] T. Miyata et al., Biochim. Biophys. Acta, 229 (1991) 672–680.
[11] S. Sakura, D. Fujimoto, Can. J. Biochem. 60 (1982) 525–529.
[12] D. Fujimoto, K. Akiba, N. Nakamura, Biochem. Biophys. Res. Commun. 76(4) (1977) 1124.
[13] Y. Kato, S. Uchida, S. Kawakishi, J. Agric. Food. Chem. 40 (1992) 373–379.
[14] A. Kamińska, A. Sionkowska, Polimery 39 (1994) 758–762.
[15] J.M. Menter, G.D. Williamson, K. Moore, I. Willis, Photochem. Photobiol. 57 (1993) 80.
[16] J. Ramshaw, L. Stephens, P. Tullock, Biochim. Biophys. Acta 1206 (1994) 225–230.
[17] Y. Kato, K. Uchida, S. Kawakishi, Photochem. Photobiol. 59(3) (1994) 343.
[18] J.M. Menter, J. Willis, Pigm. Cell. Res. 10(4) (1997) 214.
[19] A. Kamińska, A. Sionkowska, J. Photochem. Photobiol. A: Chem. 96 (1996) 123.
[20] R. Liss, Method for Serum Free Culture of Cells, Academic Press, 1984, pp. 81.
[21] E.W.J. Teale, G. Weber, Biochem. J. 65 (1957) 476.
[22] J.M. Menter, G.D. Williamson, C.L. Moore, I. Willis, K. Carlyle, Photochem. Photobiol. 62(3) (1995) 402.
[23] S.S. Lehrer, G.D. Fasman, Biochem. 6 (1967) 757.
[24] W.A. Prutz, J. Butler, E.J. Land, Int. J. Radiat. Biol. 44 (1983) 118.
[25] D. Creed, Photochem. Photobiol. 39 (1984) 563.
[26] Y. Kato, T. Nishikawa, S. Kawakishi, Photochem. Photobiol. 61(4) (1995) 367.
[27] C.S. Foote, Y.C. Chang, R.W. Denny, J. Am. Chem. Soc. 92 (1970) 5219.
[28] U.P. Andley, B.A. Clark, Photochem. Photobiol. 50 (1989) 97.