ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 96 (1996) 123–127

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A:CHEMISTRY

# Photochemical transformations in collagen in the presence of β-carotene

Alina Kamińska, Alina Sionkowska

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

Received 15 June 1995; accepted 17 November 1995

**Abstract**

The influence of UV radiation (253.7 nm) on collagen in the absence and presence of β-carotene was investigated. It was found that the relative viscosity and laevorotation of collagen decreased rapidly during irradiation. The polydispersity was also changed, and the amide bands in the IR spectra were shifted to lower frequencies. In addition, the absorbance of the $H_2O$, C=O, COOH, $COO^-$ and $CH_2$–N groups was decreased.

Such changes in the physical properties indicate that photodegradation, phototransformation and changes in the conformational state of collagen take place during UV irradiation. Smaller changes in the above properties in the presence of β-carotene suggest that it makes collagen more resistant to the action of UV radiation.

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

## 1. Introduction

Collagen is one of the most important proteins in living organisms. It is found in the connective tissue, internal organs, blood vessels and skin. Intrachain and interchain hydrogen bonds in the polypeptide chains of collagen lead to the formation of a triple helix. However, the extent of this arrangement is different for different types of collagen and depends on the function performed by this protein [1].

Collagen often exists in organisms in the liquid crystalline state. The degree of arrangement of the molecules in this state is very sensitive to the influence of various factors, such as the temperature, concentration or sun-generated radiation [2,3].

Investigations of the influence of UV radiation on the properties of collagen are rather scarce and some do not specify the radiation wavelength applied.

It has been shown that a solution of collagen, after irradiation, loses the ability to form natural fibrils [4]. In addition, the fluorescence observed after UV irradiation is due to the presence of phenylalanine and tyrosine in this protein [5–7]. Photocrosslinking [8–10] and photodegradation [7,11] of collagen may also occur on exposure to UV radiation. However, the type of photochemical process and its efficiency depend on the atmosphere [12], pH of the collagen solution [13], type of collagen and its age [14,15], light wavelength [16] and the presence of other substances in the protein [16,17].

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

## 2. Materials and methods

Collagen was obtained in our laboratory from the tail tendons of young albins rats [18]. After washing in distilled water, these tendons were dissolved in 0.04 M acetic acid solution. The samples for investigation were prepared in the form of films of 0.15 mm thickness. They were obtained by solvent evaporation from a solution of pure collagen and collagen containing 0.01% of β-carotene, poured on glass plates covered with polyethylene. The films were dried at 35 °C and preserved at room temperature and a humidity of 60%. All measurements were performed in the same conditions of temperature and humidity to avoid any influence on the position of the amide bands in the IR spectrum [19–21].

The samples in the form of films or solutions were irradiated in air at room temperature using a mercury lamp (Philips TUV-30) which emits light at mainly 253.7 nm. The intensity of radiation was 0.263 J $cm^{-2}$ $min^{-1}$.

The IR spectra of the films, before and immediately after UV irradiation, were recorded using a Carl Zeiss Specord M-20 spectrometer (Jena).

The relative viscosity of collagen solution was measured at 20 °C (0.2°) using a quartz Ubbelohde viscometer.


*SDI* 1010-6030(95)04278-4

Optical rotation was measured at 20 °C (0.1°) in a 5 cm water-jacketed tube with a Carl Zeiss Polamat A polarimeter (Jena) with an oscillating polarizer prism. Readings were taken at 546 nm.

GPC analysis was performed with a Spectra Physics SP 8810 chromatograph, equipped with a refractometer detector (Shodex RI SE-61). The column was equilibrated in water and eluted at a flow rate of 1 ml $min^{-1}$. The injection volume was 25 μl. The data were obtained using the program of PL Logical GPC/SEC.

The UV–visible spectra of collagen were recorded in air at room temperature with a Carl Zeiss M-20 spectrometer (Jena). The increase in the absorbance coefficient $\Delta a$ with increasing radiation dose was determined at 270 nm using the relationship

$$\Delta a = \frac{A_t - A_0}{d}$$

where $A_0$ is the absorbance of the non-irradiated sample, $A_t$ is the absorbance of the sample after irradiation and $d$ is the thickness of the sample (15 μm).

Electron spin resonance (ESR) spectra were recorded with an X-band spectrometer (Bruker Physik AG, B-ER-418S) operating with a 100 kHz field modulation. The spectrometer was additionally equipped with a nuclear magnetic oscillator (B-H-12) to measure the magnetic induction $B$ and a Hewlett Packard 5260A frequency divider for the determination of the frequency $\nu$. Experimentally found $B$ and $\nu$ values were used to determine the $g$ factor from the relationship

$$h\nu = g\beta B$$

where $h$ is Planck's constant and $\beta$ is the Bohr magneton. The accuracy of the $g$ factor determination was $5 \times 10^{-5}$.

The amount of free radicals in the samples was determined using the standard 1,1-diphenyl-2-picrylhydrazyle in which the concentration of paramagnetic centres is known. The accuracy of the determination of the spin concentration in the standard and the accuracy of the line recording of the sample contribute to the error in the determination of the radical concentration; the error was 6%.

## 3. Results and discussion

On UV irradiation, the relative viscosity ($\eta_{rel}$) of collagen decreases rapidly, and then remains at a stable low level without any changes with further irradiation (Fig. 1, curve 1).

In the presence of β-carotene, the change in $\eta_{rel}$ occurs slowly (Fig. 1, curve 2) and, for the irradiation time used, does not reach a stable state. This suggests that β-carotene increases the photochemical stability of this protein and hinders the processes which lead to the decrease in $\eta_{rel}$, i.e. photodegradation (with scission of bonds in the main chains) or phototransformation (with changes in the conformation of collagen molecules).

This is confirmed by GPC analysis (Figs. 2 and 3) which characterizes the polydispersity of collagen. The shapes of curves 1 and 2 (Fig. 2) are very different. The maximum of curve 2, which represents collagen after irradiation, in relation to curve 1 is lower and shifted to the region of smaller molecular weight. This suggests that photodegradation occurs mainly in collagen of high molecular weight, leading to a decrease in the content of such molecules and, at the same time, an increase in the content of protein with a smaller molecular weight.

The difference between the shapes of curves 1 and 2 in Fig. 3 is smaller than that in Fig. 2. This represents the change in the polydispersity of collagen caused by UV irradiation in the presence of β-carotene. As can be seen, the changes in

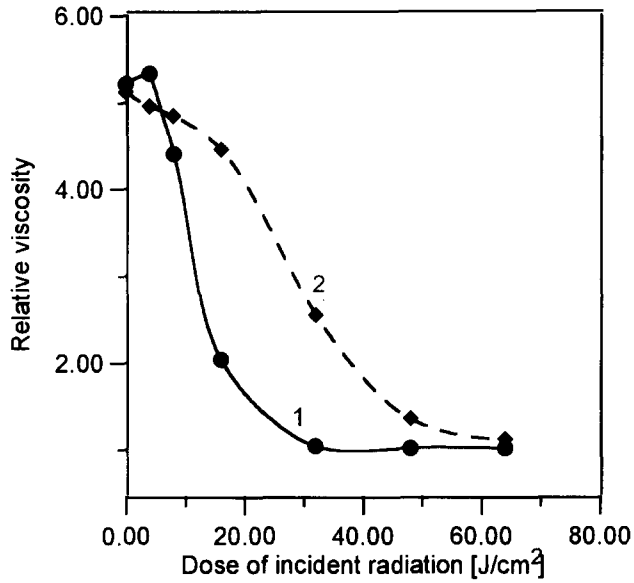



Fig. 1. Changes in $\eta_{rel}$ of collagen (curve 1) and of collagen in the presence of β-carotene (curve 2) during UV irradiation.

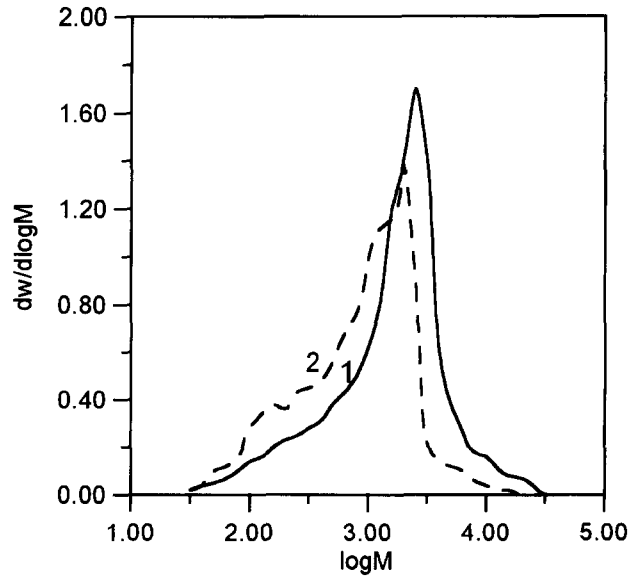



Fig. 2. Polydispersity of collagen before (curve 1) and after (curve 2) UV irradiation.

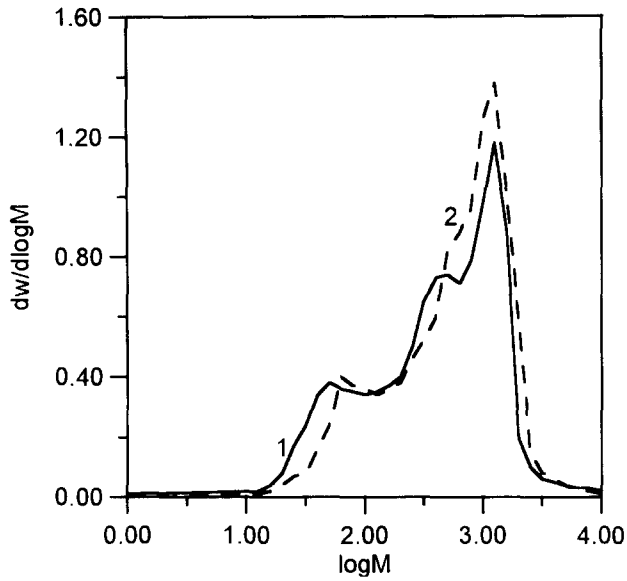



Fig. 3. Polydispersity of collagen with β-carotene before (curve 1) and after curve 2) UV irradiation.

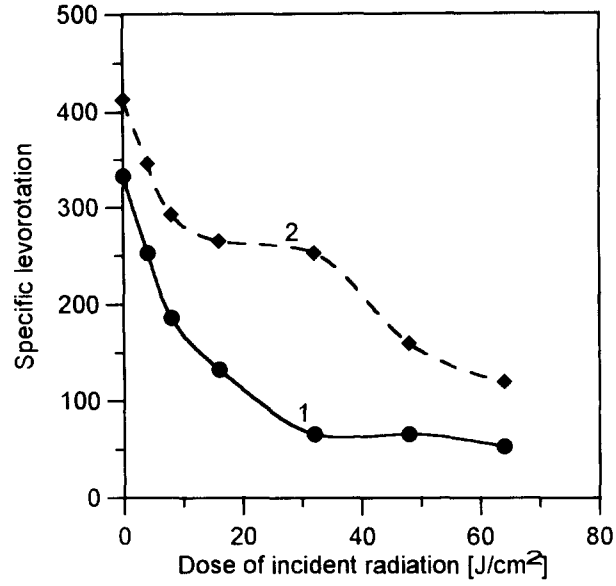



Fig. 4. Changes in the specific laevorotation of collagen (curve 1) and collagen with β-carotene (curve 2) caused by UV irradiation.

the regions of high and low molecular weights are only negligible. The small decrease in the content of low molecular weight protein and insignificant increase in the maximum in curve 2 (Fig. 3) suggest that the molecules increase in size, for example as a result of photocrosslinking.

The above results, together with the viscometric observations, suggest that β-carotene inhibits photochemical processes in collagen. However, it is not clear whether degradation or changes in configuration, or both of these photoprocesses, are responsible, because the viscosity and flow rate of protein solution through the GPC column depend not only on the molecular weight, but also on the structure of the molecules, i.e. on their hydrodynamic volume [22]. Therefore a change from a helical to a coil conformation of the protein molecules may cause a similar effect [23].

A characteristic feature of some liotropic liquid crystalline substances (i.e. those that preserve in solution, in a determined region of concentration, a structural arrangement similar to that in the crystalline state) is an especially high optical activity equal to several hundred degrees per millimetre. Such large activity is caused by the specific molecular arrangement, called cholesteric arrangement. The arrangement of molecules in the liquid crystalline state may be destroyed under the influence of temperature, pressure or radiation. As a result, many physical properties of liquid crystals may be changed. In cholesteric liquid crystals, the optical activity is changed significantly [3].

Our investigation points to a decrease in the specific laevorotation on UV irradiation in both kinds of sample (Fig. 4). However, in the sample containing β-carotene, these changes are distinctly smaller than in pure collagen. This suggests that UV radiation destroys the arranged structure of collagen and that β-carotene hampers this phototransformation.

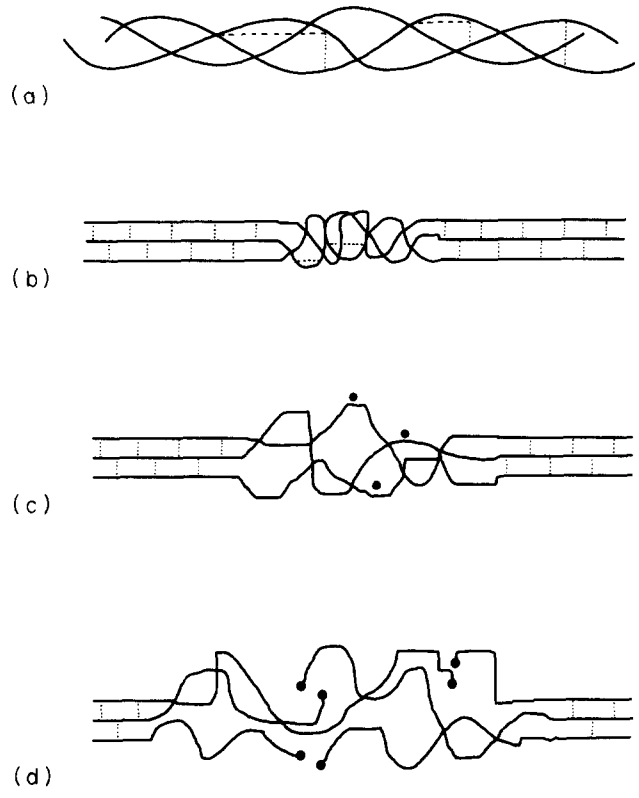
This is confirmed by an analysis of the IR spectra of collagen films before and after irradiation. In the spectra, four characteristic amide bands appear (A, B, I and II). All of these bands are shifted after UV irradiation to lower frequencies (Table 1). These shifts in the presence of β-carotene are smaller than those in pure collagen. This is especially clear for the amide A band.

Since the frequencies of the amide bands for triple helices are higher than those for random coils [21,24], the above results suggest that, on UV irradiation, phototransformation of the helical form to the coil must take place. This is possible due to the scission of hydrogen bonds on exposure to UV irradiation leading to a reduction in their number (hydrogen bonds are necessary to maintain the helical structure of collagen) (Scheme 1).

Curves 1 and 2 in Fig. 5 show the changes in the content of aromatic amino acids in the protein after denaturation. As can be seen (Fig. 5), the content of these amino acids increases during the UV irradiation of collagen. This indicates that photodenaturation, i.e. the transformation of the helical form to the coil, takes place on UV irradiation of collagen, but only slowly in the presence of β-carotene.

Table 1
Changes in the frequencies of the amide bands of collagen films caused by UV irradiation

| Band | Frequency ($cm^{-1}$) | Change in frequency $\Delta\nu$ ($cm^{-1}$) | |
|---|---|---|---|
| | | Collagen | Collagen + β-carotene |
| Amide A | 3336 | 8 | 3 |
| Amide B | 3082 | 4 | 3 |
| Amide I | 1661 | 5 | 4 |
| Amide II | 1547 | 2 | 0 |




Scheme 1.

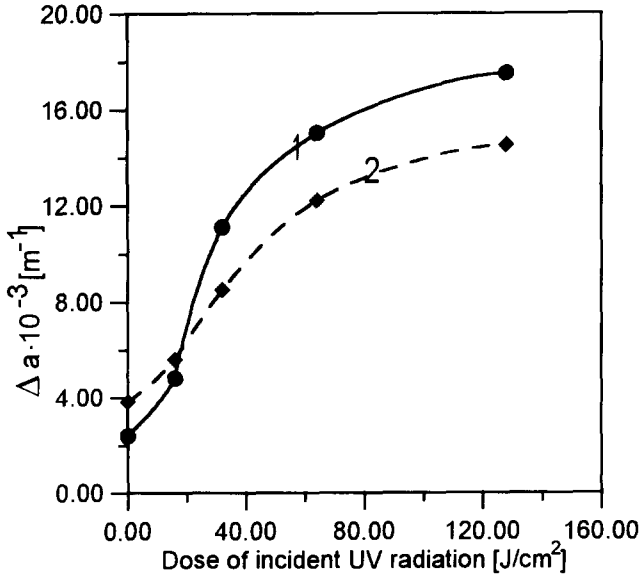
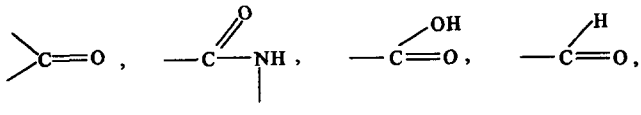



Fig. 5. Changes in the absorbance coefficient $\Delta a$ at 270 nm during irradiation of collagen (curve 1) and collagen with β-carotene (curve 2).

The above-mentioned reactions may be accompanied by the release of water linked to the protein by hydrogen bonding. This is confirmed by the decrease in the integral absorbance of the $H_2O$ band (Table 2).

It is generally known that carbonyl groups

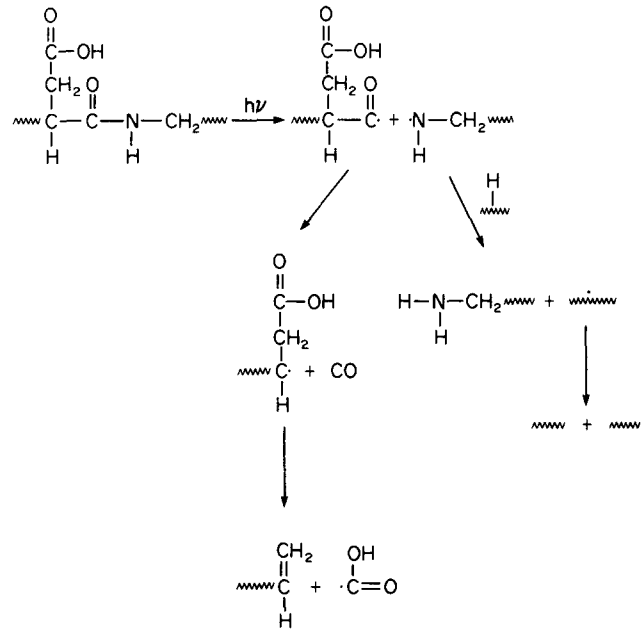


are sensitive chromophores which absorb light at wavelengths above 300 nm and may initiate the photodegradation of polymer chains and photodestruction via the cleavage of side groups [25] (Scheme 2).

The concentration of radicals formed during the first stage of the process generally determines the efficiency of the subsequent reactions. The results of ESR investigations (Fig. 6) indicate that, on UV irradiation, more radicals are created in pure collagen films than in those containing β-carotene, and larger negative changes in the integral absorbance of C=O,

Table 2
Changes in the integral absorbance of collagen and collagen with β-carotene after UV irradiation

| Assignment | Band position ($cm^{-1}$) | Changes in integral absorbance $\Delta A$ ($cm^{-1}$) | |
|---|---|---|---|
| | | Collagen | Collagen + β-carotene |
| $H_2O$ bonded | 3450 | −12.9 | −3.9 |
| $-CH_2-N$ | 3180 | −5.8 | −2.9 |
| COOH | 1710 | −0.6 | −0.4 |
| C=O | 1675 | −2.1 | −0.6 |
| $COO^-$ | 1570 | −12.8 | −4.9 |




Scheme 2.

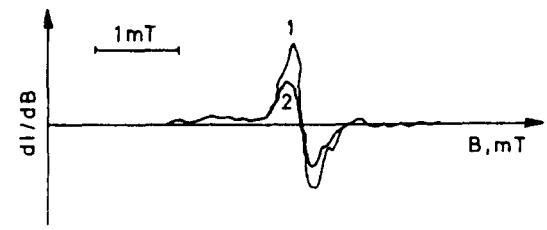



Fig. 6. ESR signals attributed to the radicals formed after UV irradiation of collagen (curve 1) and collagen with β-carotene (curve 2).

COOH, $COO^-$ and $-CH_2-N$ take place in pure collagen films (Table 2). These results suggest that β-carotene impedes the photodegradation and photodestruction of collagen and inhibits the processes which lead to the release of water from collagen, i.e. β-carotene acts as a photostabilizer of collagen.

β-Carotene, owing to its highly conjugated double bond system, absorbs light; the energy may be consumed by cis–trans izomerization of the β-carotene molecule [25–27]. At the low concentrations of β-carotene applied in our study, it was not possible to identify isomerization.

## 4. Conclusions

β-Carotene acts as a photostabilizer of collagen. This dye decreases the structural changes of collagen caused by irradiation at 253.7 nm.

## References

[1] R. Van der Rest and M. Garrone, *FASEB J., 5* (1991) 2814–2823.
[2] Ju.B. Amierik and B.A. Krienciel, *Chimia Zidkich Kristallov i Miezomorfnych Polimernychsystem*, Nauka, Moscow, 1981.
[3] P.J. Flory, *Liquid Crystal Polymers I*, Springer-Verlag, Berlin, 1984.
[4] E. Fujimori, *Biopolymers, 3* (1965) 115.
[5] E. Fujimori, *Biochemistry, 5* (1966) 1034.
[6] D.V. Crabtree and E. Fujimori, *Biopolymers, 19* (1980) 1081.
[7] E. Fujimori, *Eur. J. Biochem., 152* (1985) 299–306.
[8] Y. Kano, Y. Sakano and E. Fujimoto, *J. Biochem., 102* (1987) 839.
[9] E. Fujimori, *FEBS Lett., 253* (1988) 98.
[10] A.J. Gasan, *Biofizika, 33* (1988) 772.
[11] S.F. Curran, M.A. Amoruso and D.B. Goldstein, *FEBS Lett., 76* (1981) 155.
[12] T. Miyata et al., *Biochim. Biophys. Acta, 229* (1991) 672–680.
[13] S. Sakura and D. Fujimoto, *Can. J. Biochem., 60* (1982) 525–529.
[14] D. Fujimoto, K. Akiba and N. Nakamura, *Biochem. Biophys. Res. Commun., 76* (4) (1977) 1124.
[15] Y. Kato, S. Uchida and S. Kawakishi, *J. Agric. Food. Chem., 40* (1992) 373–379.
[16] A. Kamińska and A. Sionkowska, *Polimery, 39* (1994) 758–762.
[17] J. Ramshaw, L. Stephens and P. Tullock, *Biochim. Biophys. Acta, 1206* (1994) 225–230.
[18] R. Liss, *Method for Serum Free Culture of Cells*, Academic Press, 1984, p. 81.
[19] A. Huc and J. Sanejanoud, *Biochim. Biophys. Acta, 154* (1968) 408.
[20] B. Brodsky-Doyle, E.G. Bendit and E.R. Blout, *Biopolymers, 14* (1975) 937.
[21] Yu.A. Lazarev and A.V. Lazareva, *Biopolymers, 17* (1978) 1197–1214.
[22] P.J. Flory, *Principles of Polymer Chemistry*, Cornell University, Ithaca, New York, 1953.
[23] P.J. Flory, *J. Polym. Sci., 48* (1961) 105.
[24] T. Miyazawa, *J. Chem. Phys., 35* (1961) 693–713.
[25] J.F. Rabek, *Mechanisms of Photophysical Processes and Photochemical Reactions in Polymers*, Wiley, New York, 1987, p. 640.
[26] C.S. Foote, Y.C. Chang and R.W. Denny, *J. Am. Chem. Soc., 92* (1970) 5219.
[27] J.F. Rabek and D. Lala, *J. Polym. Sci., Polym. Lett., 18* (1980) 427.