Effect of Ultraviolet Radiation on Photodegradation of Collagen

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ABSTRACT: Photodegradation of solvent-cast collagen type I films and photostabilization of collagen by vitamin E were studied. These films were exposed to polychromatic radiation from a medium-pressure mercury lamp or monochromatic radiation from the Okazaki Large Spectrograph (OLS). Changes in the molecular structure of collagen were followed by UV-visible and FTIR spectroscopic measurements. Electron spin resonance (ESR) spectroscopic measurements were also carried out to identify the reaction intermediates of photodegradation. Photoreaction from phenylalanine, which is one of the main constituents of collagen to tyrosine and the scission of peptide linkage of collagen, were confirmed. Vitamin E was found to be an efficient photostabilizer for photodegradation of collagen. Action spectra of photodegradation and photostabilization of a collagen molecule were reported. A possible mechanism for the photodegradation of the collagen and photostabilization scheme based on these experimental results are presented. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 73: 1259–1265, 1999

Key words: ultraviolet radiation; photodegradation of collagen; solvent-cast collagen type I films

INTRODUCTION

Collagen is one of the popular proteins consisting of various kinds of amino acids, such as glycine, alanine, proline, and phenylalanine. Collagen is one of the constituents of animal and human skin, bone, tendon, teeth, nail, and cell walls, and has a supercoiled triple helix structure. Photoaging of collagen by UV-A or UV-B radiation is well known. It is said that the insoluble fraction in collagen increases with the increase of the age because of photocrosslinking reaction in collagen. The photodegradation of collagen may result from denaturation of protein and/or photocrosslinking between polymer molecules. Changes in the molecular structure may induce some damage on human skin and other organs.

Recently, a stratospheric ozone depletion has been observed on the earth, and this induces the enhancement of UV-B radiation reaching the earth's surface. The UV-B radiation is reported to cause skin cancer and/or other diseases by the damage of the collagen molecule. It is important to know the photodegradation mechanism of collagen from these view points. Under these considerations, we have studied the wavelength sensitivity of photodegradation of collagen to chemically clarify the reaction mechanism chemically.

We also investigated the effect of vitamin E on the photodegradation of collagen to find out the effective quencher against photoinduced damage of collagen.

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EXPERIMENTAL

Preparation of Collagen Film

Commercial collagen type I powders from calf skin purchased from the Nacalai Tesque Corporation were dissolved into a 5% acetic acid solution with stirring overnight. The concentration of collagen in the acetic acid solution was approximately 1 wt %. The solution was cast on a clean surface of mercury. The collagen films thus obtained were wiped with ethyl alcohol and preserved at an ambient temperature and in the dark. Vitamin E was added to the acetic acid solution of the collagen (the concentration of vitamin E in acetic solution is 0.029 mol/kg) and additive-containing films were obtained by the same procedure as described above. Translucent films of collagen and collagens containing vitamin E were obtained by this method and the thickness of the films was around 0.03–0.08 mm.

Polychromatic and Monochromatic Exposure Studies

Sample films were irradiated with a Toshiba H-400P medium-pressure mercury lamp ($\lambda \geq 250$ nm) in air at 45°C. The distance between the light source and the sample was 15 cm, and the radiation intensities at the sample positions were 820 w/m^2.

Monochromatic irradiation to the sample films was carried out using the Okazaki Large Spectrograph (OLS), which gives the monochromatic radiation of any desired wavelength between 250 and 1000 nm with high radiation intensity. The design and the features of this spectrograph have already been reported.[1]

The beam was focused on the surface of the samples by using a surface mirror (20 × 10 cm). A schematic representation of the monochromatic radiation exposure was also given in our previous article.[2,3] Samples were irradiated at eight different wavelengths: 275, 295, 305, 320, 335, 350, 365, and 380 nm at 23°C in air. The radiation intensity at each sample position was measured by a PFDM-200LX photon density meter that was provided from the Rayon Co.

The total photons per square cm of the sample was 3.62×10^{19} photons/cm^2 at each wavelength.

Spectroscopic Studies

UV-visible spectra of photoirradiated samples were measured on a Jasco V-550 UV/VIS spectrophotometer. A Jasco 5300 FTIR spectrophotometer was used to measure FTIR spectra of photoirradiated samples. Reaction intermediates in photoirradiated samples were analyzed with ESR spectra that were taken by a JEOL-3BX ESR spectrometer. ESR studies were limited to the polychromatic exposure study. Irradiations were carried out at 43°C in air, placing the samples in a quartz tube.

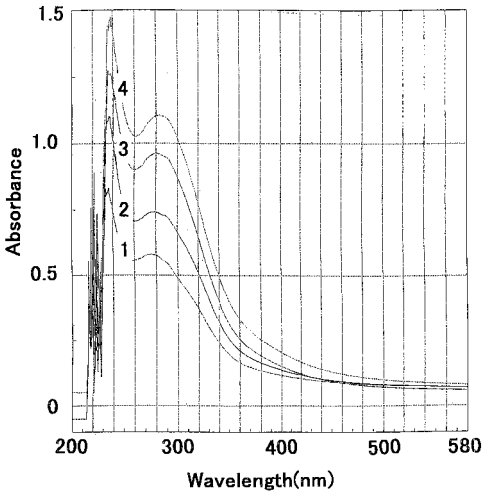


Figure 1 UV difference spectra of photoirradiated and unirradiated collagen film at different irradiation times: 1, 1 h; 2, 2 h; 3, 3 h; 4, 4 h.

RESULTS AND DISCUSSION

Polychromatic Exposure Studies

Collagen film has the absorption maxima at 280 nm and a shorter wavelength than this band. These bands increased in the intensity upon photoirradiation. The difference spectra between photoirradiated and unirradiated collagen film are shown in Figure 1. As seen from the absorption spectra in Figure 1, the increase in the intensity at 235 and 280 nm is confirmed.

The increase in intensity at 235 and 280 nm bands is attributed to the decay of the peptide linkage and aromatic amino acid absorption,[4] respectively. The intensity of the band at 280 nm markedly increases, and the absorption maximum shifts to a longer wavelength with the increase of the irradiation time. The increase in the intensity at 280 nm is plotted against the irradiation time in Figure 2.

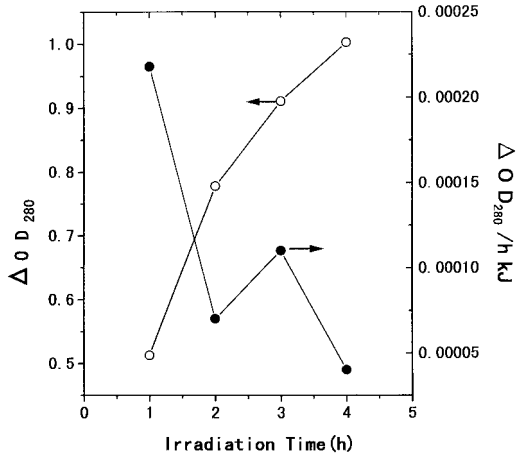
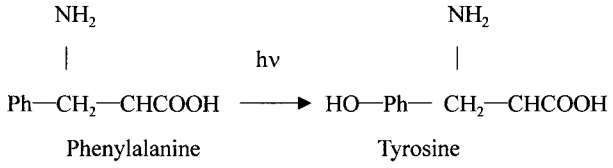


Figure 2 Changes in optical density at 280 nm and rate of increase in optical density at 280 nm with irradiation time.

At the same time, the rate of increase in the intensity at 280 nm (given as the change in the intensity per unit photon intensity and time) is also plotted against the irradiation time (also in Fig. 2). From this figure it is evident that the rate of the reaction decreases with the increase of the irradiation time.

The increase in the intensity and the red shift of the 280 nm bands suggests that the decrease of phenylalanine and the formation of tyrosine in the collagen molecule. Tyrosine has an absorption maximum at 278 nm, and the molar extinction coefficient (ε) of tyrosine is about seven times that of phenylalanine.[5,6]

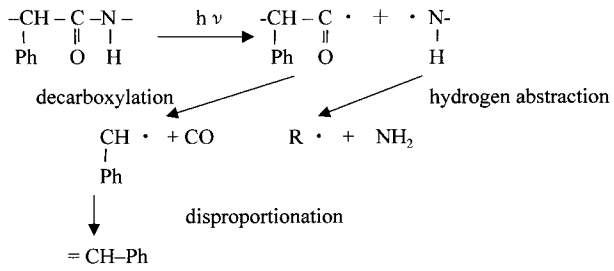
These increases may be attributed to the formation of tyrosin from phenylalanine for the 280-nm band and the decrease of the peptide linkage by photoirradiation for 235 nm.



Formation of tyrosine is said to be an initial step for the photoaging of animal skin.[7]

FTIR spectra of photoirradiated collagen were given in Figure 3.

The amide A (3350 cm^{-1}), amide B (3100 cm^{-1}), amide I (1650 cm^{-1}), and amide II (1550 cm^{-1}) bands are related to the peptide linkage of the collagen.[8] These bands decreased in their intensities, with the irradiation time showing the degradation of the peptide linkage. From the experimental results obtained, the following reaction scheme is proposed.



The wave numbers of amide I and amide A bands shift to the longer wave number with the irradiation time, as shown in Figure 4.

This shift in the wave number is concerned with the destruction of the hydrogen bonds, which are formed between the helix of the collagen molecule, and shows the partial destruction of the triple helix structure of the collagen molecule to the random coil structure. This transformation was also reported for UV-irradiated collagen from a rat tail tendon.[9]

ESR spectra of photoirradiated collagen and collagen containing vitamin E as an additive were analyzed to see the effect of vitamin E on the photodegradation of collagen. The spectra were shown in Figure 5.

The signal produced by photoirradiation is lower in the case of the collagen containing vitamin E as an additive at any irradiation time compared. The relative radical amounts (indicated as relative ESR peak height) of photoirradiated col-

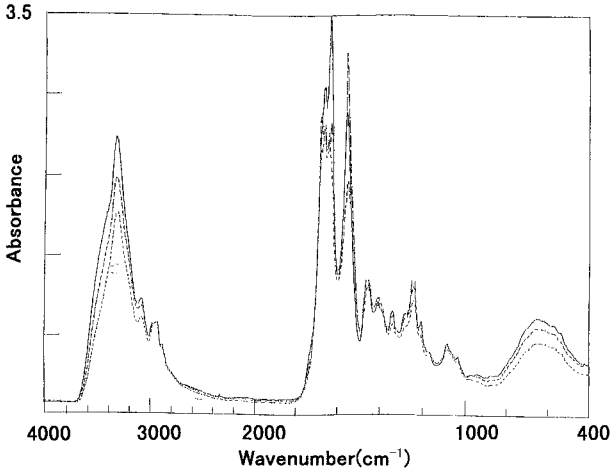


Figure 3 FTIR spectra of photoirradiated collagen films. Irradiation time, ——, 0 h; - - - - -, 3 h; · · · · · ·, 6 h.

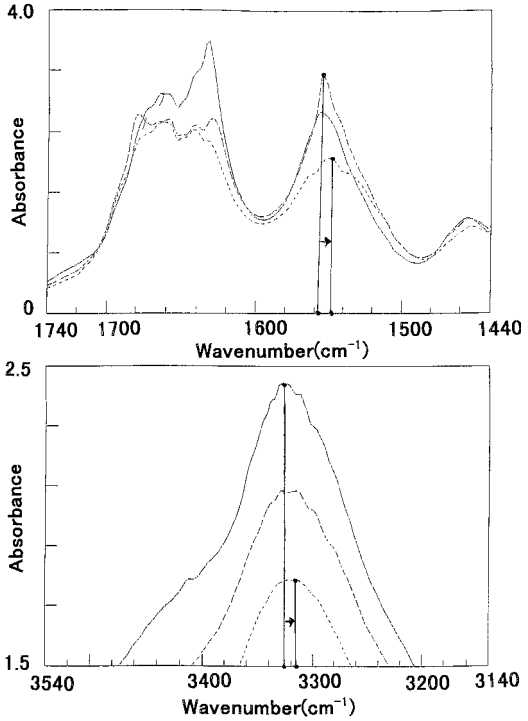


Figure 4 Extended FTIR spectra of photoirradiated collagen films. Irradiation time, ——, 0 h; - - - - -, 3 h; · · · · · ·, 6 h.

lagen and collagen containing vitamin E as an additive were plotted against the irradiation time in Figure 6.

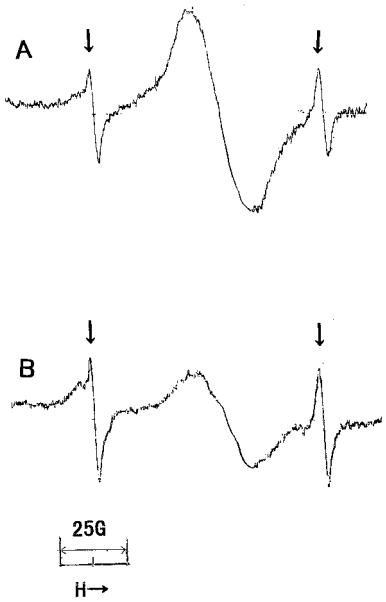


Figure 5 ESR spectra of photoirradiated (A) collagen, and (B) collagen containing vitamin E. Irradiation time, 4 h. Arrows indicate the signal of Mn^{2+}.

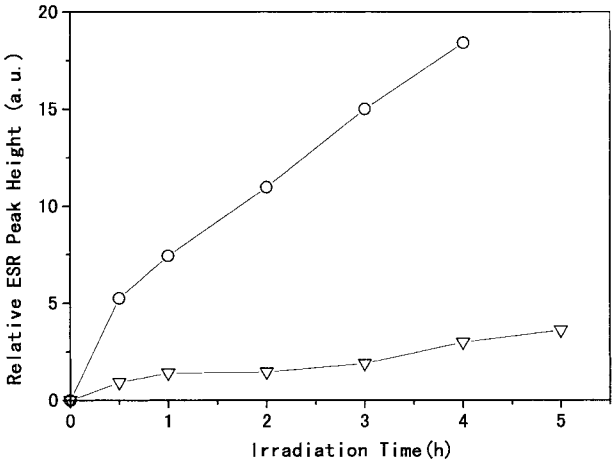


Figure 6 Relative ESR peak height of photoirradiated (○) collagen and (▽) collagen containing vitamin E.

The amount of the radical was suppressed by the addition of vitamin E. This result shows that vitamin E can suppress the photodamage of the collagen.

This fact is also confirmed by UV-spectral measurements. As shown in Figure 7, the increase in the O.D. at 280 nm was suppressed by the addition of vitamin E.

As we have discussed, formation of tyrosine, which is thought to be an initial step of photodegradation of collagen, is clearly suppressed by the addition of vitamin E to the collagen film.

The results obtained from the ESR and UV-spectral measurement show that the photodegradation of the collagen is efficiently suppressed by the addition of a small amount of vitamin E.

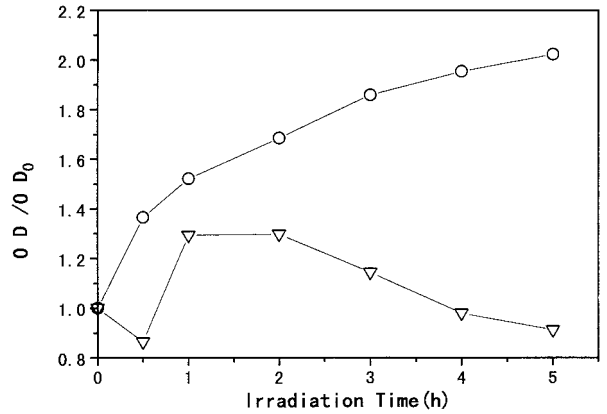


Figure 7 Changes in optical densities at 280 nm with irradiation time. ○, collagen without additive; ▽,collagen containing vitamin E.

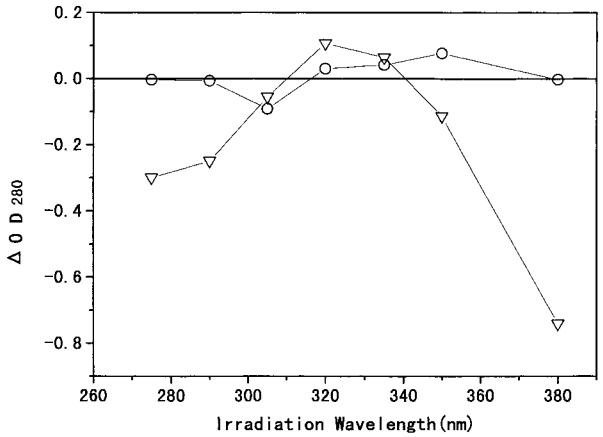


Figure 8 Changes in optical density at 280 nm with irradiation wavelength. ○, collagen without additive; ▽, collagen containing vitamin E.

This fact may show that vitamin E is an effective stabilizer for photoaging of animal skin.

Monochromatic Exposure Studies

Wavelength sensitivity of the photodegradation of collagen films were studied by UV-spectra and FTIR spectra. On exposure to monochromatic radiation (approximately 3.6×10^{19} photons/cm^2 at each each wavelength), the absorption band at 280 nm changes in its intensity. ΔO.D. at 280 nm, which shows the difference between the optical density at 280 nm before and after photoirradiation, changed, depending on the irradiation wavelength. Changes in ΔO.D. at 280 nm are plotted against the irradiation wavelength in Figure 8, along with the samples containing vitamin E as an additive.

The effect of the wavelength on ΔO.D. depends on the irradiation wavelength. ΔO.D. decreases in its intensity at the irradiation wavelength of 305 nm. This decrease in intensity may be attributed the photoreductive reaction from tyrosine to phenylalanine. On the contrary, ΔO.D. increases in its intensity at the irradiation wavelength at 350 nm. This increase may arise from the photo-oxidative reaction from phenylalanine to tyrosine. Upon the addition of vitamin E as an additive, ΔO.D. decreases at all irradiation wavelengths except for 320 and 335 nm. This decrease may be attributed to the suppression of the photooxidative reaction of collagen.

At the irradiation wavelength 350 nm, ΔO.D. increases in its intensity, showing some specific reaction concerned at this wavelength.

FTIR spectra of photoirradiated collagen and collagen containing vitamin E as an additive at various wavelengths were taken, and the changes in the optical densities at 1650 cm^{-1} (amide I band) and 1550 cm^{-1} (amide II band) were plotted against the irradiation wavelength (Fig. 9).

The optical densities of these bands decrease in their intensities at each irradiation wavelength except for 275 and 320 nm. This decrease means that the peptide linkage of the collagen molecule was broken by the photoirradiation wavelength dependently, as in the case of the irradiation with polychromatic radiation.

Adding vitamin E as an additive, this decrease is suppressed efficiently at all wavelengths irradiated. Although the specific dependence on the irradiation wavelength could not be elucidated in this stage, the photoprotection against the collagen molecule by vitamin E was also confirmed in the monochromatic radiation exposure measurements.

The protection reaction against the photodegradation of collagen by vitamin E is clearly demonstrated from the experimental results. The following two processes for the protection reaction of collagen from UV radiation will be considered.

Vitamin E as a UV Absorber

Vitamin E (Vit E) absorbs UV-radiation, the Vit E radical produced by photoabsorption produces stable quinone in a short time, and the Vit E radical disapears. Photoabsorption by collagen is reduced by this process, and the resulting degra-

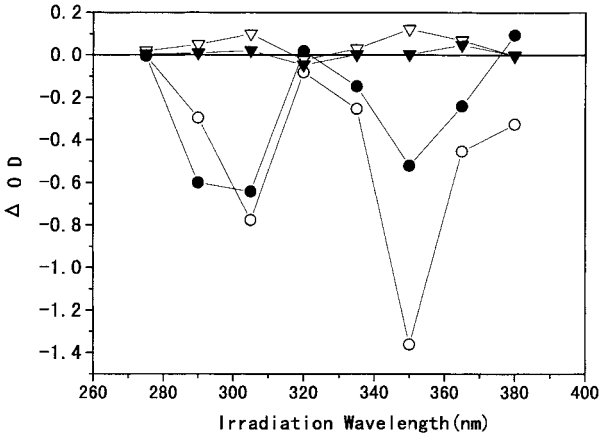


Figure 9 Changes in optical densities at 1650 cm^{-1} (○, ▽) and 1550 cm^{-1} (●, ▼) with irradiation wavelength. ○, ●, collagen without additive; ▽, ▼, collagen containing vitamin E.

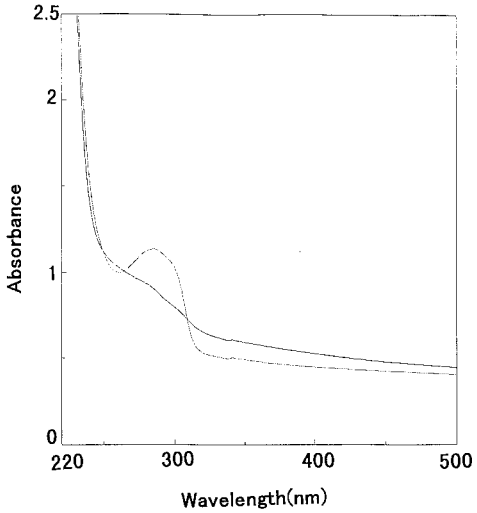


Figure 10 Absorption spectra of collagen containing vitamin E · · · · · ·, unirradiated; ——, photoirradiated 2 h.

dation of collagen is suppressed in the presence of vitamin E. The processes may be written as follows.

$$\text{Vit E} \xrightarrow{h\nu x_1} \text{Vit E} \cdot \longrightarrow \text{Quinone type compound}$$

$$\text{Phenylalanine (Phe)} \xrightarrow{h\nu x_2} \text{Tyrosine}$$

where, $(h\nu x_1 + h\nu_2)$ indicates the total photon energy provided to this system. The initial step to produce tyrosine from phenylalanine is suppressed by the decrease of photoabsorption with Vit E.

Vitamin E as Radical Scavenger

$$\text{Phe} \xrightarrow{h\nu} \text{Phe} \cdot$$

$$\text{Phe} \cdot + \text{Vit E} \longrightarrow \text{Phe} + \text{Vit E} \cdot \downarrow \text{Stable compound}$$

Phenylalanine radical produced by photoabsorption is scavenged by Vit E, and the Vit E radical converts to a stable product such as a quinone-type compound.

A UV spectrum of photoirradiated collagen film is shown in Figure 10.

The absorption band at 295 nm arising from collagen containing vitamin E decreases in its intensity by photoirradiation, and at the same time ΔO.D. at 280 nm decreases by the addition of vitamin E to the collagen. This fact means that photoabsorption by vitamin E can suppress the formation of the tyrosine molecule from phenylanine in the photodegradation of collagen.

Thermal-Induced Changes in Collagen

It is said that although collagen has an excellent property to retain moisture in the molecule, the moisture retained in collagen is lost by thermal treatment. As a result, some chemical changes in the collagen molecule may be induced. FTIR and UV spectra of thermal-treated collagen were taken to analyze the changes in chemical structure at several temperatures. FTIR difference spectra of thermal-treated collagen at 47, 87, and 117°C are shown in Figure 11.

On irradiation at 47°C, the absorption band at about 1650 cm^{-1} increased.

With raising the temperature, the intensity of this band and the band around 1550 cm^{-1} in-

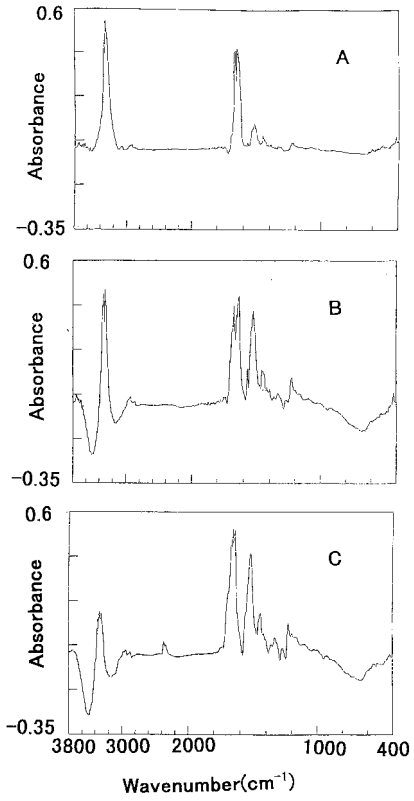


Figure 11 FTIR difference spectra of thermal-treated collagen (A) at 47°C, (B) at 87°C, and (C) at 117°C.

creased in their intensities. When the samples were irradiated at a higher temperature, the band at around 3500 cm^{-1} that related to the OH bond of the water molecule decreases in its intensity. This fact shows the decrease of moisture form the collagen molecule. UV spectra of heat-treated collagen show an increase of the intensity at 235 nm, showing an increase of the peptide linkage. The band at around 280 nm did not change in its intensity. From UV spectral measurement, it is clear that photoirradiation is a prerequisite for tyrosine formation.

CONCLUSIONS

Photodegradation of collagen results in the scission of peptide linkage and tyrosine formation from phenylalanine, which is one of the main constituents of collagen by an oxdative reaction. The photoirradiation is a prerequisite for the tyrosine formation in collagen.

These reactions are suppressed by the addition of vitamin E to collagen.

Wavelength sensitivity of the photodegradation are also studied, and the specific dependence to the irradiation wavelength was found.

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