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ScienceDirect
Journal of Photochemistry and Photobiology A: Chemistry 188 (2007) 192–199
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Spectroscopic study of a KrF excimer laser treated surface of the thin collagen films

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Received 11 August 2006; received in revised form 13 November 2006; accepted 8 December 2006
Available online 14 December 2006

Abstract

The photochemical properties of collagen films before and after KrF excimer laser UV-irradiation with 248 nm were investigated using Raman, FTIR-ATR and fluorescence spectroscopy. It was shown that a single pulse of UV radiation ($\lambda = 248$ nm) can affect the conformation and photostability of collagen polypeptide chains. Raman and FTIR-ATR spectra analysis showed that UV laser light is capable of inducing conformational changes in the irradiated collagen films, mainly as a result of breaking of the hydrogen bonds network and losing of water molecules, accounting for the maintaining the structure organisation. Partial decomposition of the main collagen chain is also considered. Fluorescence measurements showed characteristic bands assigned to tyrosine aromatic compound and also to the products of its photochemical degradation given by laser irradiation.

The obtained results indicate that the interaction between collagen film and UV laser radiation can be considered as the result of the photomechanical regime, with low thermal degradation, combined with some photochemical transformations.

Keywords: Collagen film; "Micro-foam" structure; Triple helix; Random coil; Raman spectroscopy; FTIR-ATR spectroscopy; KrF excimer laser; Laser radiation

1. Introduction

Collagen has outstanding molecular architecture and properties and it is a suitable material for laser–matter interaction research [1,2]. Collagen in the human body accounts for about 25% of all proteins and it is the main component of connective tissue, occurring in skin, tendons, cornea, bone and membranes [1–3]. It is a strongly hydrophilic protein that explains the ability of collagen materials to bind a large amount of water in its internal structure [3–6]. The newest investigations show that in the collagen family 20 types of collagen are known [7,8]. The triple helix structure is maintained by hydrogen bridges between –NH group of glycine and carbonyl group C=O of residues from another polypeptide chain or by hydrogen bridges with water molecules [9–11]. Moreover, the influence on stabilization of the collagen helix is also due to short-range interactions such as: Van der Waals, hydrophobic or electrostatic [3,12]. In fibrillar collagens, ~25% of the amino acids in the α-chain are proline or hydroxyproline that effectively block internal rotation of the collagen chain at these sites and stabilize further the triple-helical structure [4]. Because of its interesting biological properties like non-toxicity and its large availability, collagen is extensively used as a source biomaterial in medicine, pharmaceutical and cosmetic industries [13]. After extraction from natural tissue, collagen materials can be processed to obtain thin films, sponges, membranes and fibres.

In our recent papers we presented a new laser processing of the studied biopolymer film surfaces, collagen, collagen/PVP blend and chitosan. A single KrF laser pulse of sufficient energy density for ablation excites the skin depth of the film (~15 μm) yielding a new micro-foam layer [14–19] having some promising biomimetic properties that could be used in cell culturing. This new and interesting result was unexpected since shorter but similar wavelength ArF laser at 193 nm gives only the traditional

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doi:10.1016/j.jphotochem.2006.12.012

clean ablation which is now extensively being used in refractive cornea surgery. Studies of laser treatment at 248 nm are scarce because of the low absorptivity of cornea, a collagen rich tissue, which does not permit the so called clean ablation. However, we demonstrate with dry collagen films new interesting phenomena are observed at this wavelength.

In this framework we show the way in which single pulses of UV radiation ($\lambda = 248$ nm) can affect the conformation and photostability of collagen polypeptide chains. We will try to answer the question whether a partial loss of the unique three-dimensional protein structure, the triple helix, could be explained by means of known ablation mechanisms and other one photon photochemistry. The laser induced micro-foam structure was therefore investigated by several methods, including vibrational and electronic spectroscopies with the sake of tracking of the possible molecular damage and alteration to the molecular structure of collagen.

2. Experimental approaches

2.1. Materials

Collagen (Type I) was obtained in our laboratory from the tail tendons of young albinos rats. After washing in distilled water, tendons were dissolved in 0.4 M acetic acid solution. Non-dissolved parts of tissue were separated by centrifugation at 7500 rpm. Thin biopolymer films, of ~35 μm thickness, were obtained by casting the prepared aqueous solution onto glass plates and drying in air at room temperature overnight. The films under the study contain only molecules in both triple-helical and random coil conformations. They do not contain fibrils, as we could not find any fibres using X-ray diffraction methods [20].

2.2. Methods

2.2.1. Laser system

The source of UV radiation was krypton-fluoride (KrF) excimer laser (Lambda Physik, LPX 220i) emitting the wavelength of 248 nm and pulse duration of 25 ns, with a method of beam shaping as described in a recent paper [14]. The irradiation was carried out in air and at room temperature, by using single pulses of UV light isolated out manually from the output of the laser working at a repetition rate of 1 Hz.

2.2.2. Raman spectroscopy

Raman spectra of collagen films before and after laser irradiation (fluence 1.0 J/cm^2, 1 pulse) were recorded in air at room temperature using a Confocal Raman Microspectrometer (Horiba Group), equipped with an argon laser delivering 10 mW of power at the wavelength of 514.5 nm. Data collection and plots were achieved with the LabSpec program supplied by manufacturer.

2.2.3. FTIR-ATR spectroscopy

Infrared spectra of the examined collagen films were recorded in the 500–4000 cm^{-1} window using Nicolet Magna IR 560 spectrometer with a probe cell equipped with a diamond crystal prism working in the attenuated total reflection (ATR) mode. These measurements could be done up to 5 pulses and were not measurable for more because of the too large surface roughness which is increasing with delivered pulses number. All spectra were acquired in air at room temperature and each measurement was an average of 300 scans.

2.2.4. Fluorescence measurements

The intrinsic fluorescence studies were performed on a Spex Fluorolog 212 (Horiba Group) spectrophotometer. Measurements were done using mainly 270 and 350 nm excitation wavelengths, before and after laser irradiation of the collagen film surface at a fluence of 1.0 J/cm^2. Absorption and emission bands were assigned to aromatic amino acids, mainly tyrosine and to their degradation products.

3. Results and discussion

3.1. Raman spectra

The peptide bonds give rise to several classical and well documented vibrational modes in the Raman spectra which are reported as amide I–VII, A and B bands. Amide I and III bands have relatively high Raman intensity and are more sensitive to conformation change than others. These bands were analysed to determine the changes in the internal structure of the collagen molecules presented in this work. The amide I band consists of C=O, C–N stretching (approx. 80% and 10% contribution, respectively) vibrations and N–H bending vibrations, whereas the amide III band represents mainly C–N stretching, N–H bending and CH_3–C stretching vibrations (approx. 40%, 30%, 20% contribution, respectively) [21–23]. In order to determine the protein or polypeptide chain conformation using amide I and III bands, the frequencies of these bands should be related to Ramachandran angles (ψ and φ-angles of rotation) which result from the possibilities of polypeptide backbone to rotate around C_α–C and C_α–N bonds [21–24]. The conformation of a protein molecule such as collagen is mainly determined by interaction of amide backbone with amino acid side chains, which are of steric nature. The conformation of the polypeptide chain is maintained by means of intermolecular hydrogen bonding between oxygen from the carbonyl group of each peptide bond and hydrogen atom coming from the amine group of every fourth amino acid. The internal hydrogen bonds form periodically within the same main chain at each turn of the helix [22,24]. Establishing the conformational changes in the protein molecule (for example: conversion from helical to random coil structure) based on Raman spectra obtained, should include an interpretation of the two amide bands (I and III), simultaneously. Analysis of amide III band only, owing to its complexity, can lead to a false conclusion concerning structural changes in the material studied.

In Fig. 1 the Raman spectra obtained for the non-irradiated collagen film and the sample irradiated with a single laser pulse at a fluence of 1.0 J/cm^2 are shown. The main bands seen in the spectra are located at the following wavenumbers: 1669–1638, 1454, 1265–1244, 1004, 937–921, 876–855 and 815 cm^{-1}. A

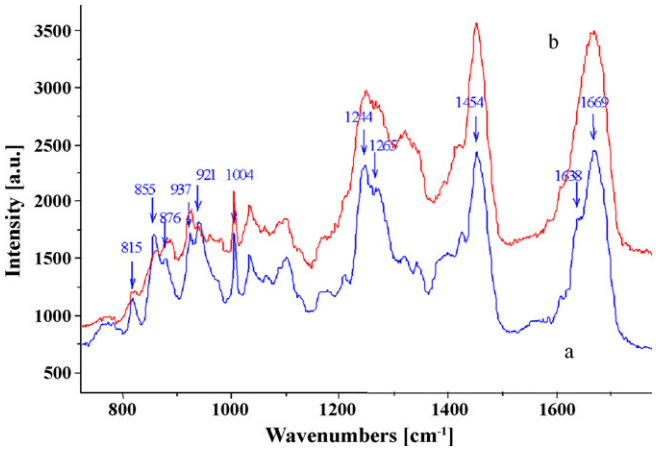


Fig. 1. Raman spectra for collagen film surface before (blue line-a) and after laser pulse at a fluence of 1.0 J/cm^2 (red line-b). In the spectrum the characteristic bands of collagen were marked.

summary of the attributions is proposed in Table 1. Bands in the 1669–1638 cm^{-1} region are assigned to the amide I band, where the first value relates to the disordered phase attributed to random coil and the second one to the triple helix structure. In the case of the amide III group of bands in the range of 1265–1244 cm^{-1} the first wavenumber at 1265 cm^{-1} can be attributed to the triple helix structure, whereas the next one is due to the random coil structure. The bands in the range of 937–921 cm^{-1} correspond to C–N stretching vibrations in proline or the main chain vibrations of the triple helix, and the frequencies at 876–855 cm^{-1} are also assigned to proline, hydroxyproline or to the aromatic amino acid side chain of tyrosine. The remaining bands around 1454 cm^{-1} are attributed to deformation vibrations in –CH_3, –CH_2 groups, 1004 cm^{-1} stretching vibrations in phenylalanine benzene ring, and 815 cm^{-1} is the stretching vibrations in the main chain of the collagen molecule [22,25–28]. As a result of single pulse laser irradiation at a fluence of 1.0 J/cm^2 we mainly observed the disappearance of the band at 1638 cm^{-1} which is attributed to the ordered helical structure. The triple helix is maintained by means of an extended hydrogen bond network, especially between glycine amino groups of the single helix and the proline carbonyl groups in the X neighbouring position. It is also maintained by water bridges between hydroxyproline in the Y position and proline. Roughly, the overall amino acid sequence of the collagen triple helix can be described with $(Gly\text{-}X\text{-}Y)_n$, where X is proline and Y is hydroxyproline [9–11]. Thus, the disappearance of the band at 1638 cm^{-1} can be ascribed to the breaking of these bonds leading to the loss of the native structure of the collagen molecule.

As well as hydrogen bonds there also exist other non-covalent interactions such as hydrophobic or electrostatic forces accounting for spatial protein conformation. Their removal facilitates conversion to the disordered random coil structure. It can be confirmed by the changes to the amide III band at 1265 cm^{-1} which becomes smoother indicating a reduction of the ordered structure. The above-mentioned structure (random coil) seems to be confirmed by the band analysis at 937–921 cm^{-1} because this band should disappear during transition to the random structure [22].

Raman spectra of the collagen film exposed to laser light revealed changes in the 876–855 cm^{-1} region that could be explained as a change in the environment of the tyrosine side chain [22] or as a change within the hydroxyproline which is one of the main components in the collagen amino acid sequence [27,9–11]. Tyrosine is a hydrophobic aromatic amino acid and is the main chromophore of the collagen molecule. It is capable of absorbing UV light [29] including pulses generated by KrF

Table 1
List of vibration frequencies of the collagen films with tentative attribution

Position (cm^{-1})	Vibration	Raman intensity	Group attribution
3300	Fermi resonance, overtone of amide II and ν(NH)		Amide A
3078			Amide B
1669	ν(CO), δ(N–H) wag	******s	Amide I, random coil
1638	ν(CO), δ(N–H) wag	***m, sh	Amide I, triple helix and H_2O bend
1605	ν(CC) benzene ring	w, sh	Tyrosine and phenylalanine
1550	In plane NH bend coupled with ν(C–N)	Not seen	Amide II
1454	δ(CH_2 and CH_3), wag	******s	Invariable, can be used as reference
1421	νs(COO–)	***m, sh	
1321	ν(C–NH_2)		
1264	δ(NH)	*****s, sh	Amide III, triple helix
1244	δ(NH)	******s	Amide III, random coil
1163	In plane δ(CH)	w	Tyrosine
1095	ν(CN)	***m	
1032	ν(CC), benzene ring breathing	***m	Phenylalanine
1004	ν(CC), benzene ring breathing	*****s	Phenylalanine, tyrosine, tryptophane
937	ν(CC)	***m	Hyp and backbone
921	ν(CC)	**m	Pro and Hyp
876	ν(CC)	**m	Pro, Hyp ring and tyrosine doublet
855	ν(CC)	**m	Pro ring and tyrosine doublet
815	ν(COC), ν(CC)	w	Backbone

s = strong, m = medium, w = weak, ν = stretching coordinate, δ = deformation coordinate, sh = shoulder. (***) The number of stars means order of magnitude of the intensity

excimer laser (λ = 248 nm). Tyrosine is also involved in hydrogen bond formation. If we consider the changes in the tyrosine microenvironment, it is necessary to take note of the band doublet at wavenumbers 876/855 cm^{-1}, which comes into play as being the result of a Fermi resonance between a ring vibration and an overtone of bending ring vibration (out-of-plane) in *para*-substituted benzene. There are two ways of doublet interpretation [22]. One of them concerns the intensity ratio of two peaks depending whether the tyrosine side chain is "exposed" (outside main chain, $I_{855} < I_{876}$) or "covered" (within main chain, $I_{855} > I_{876}$). The latter one combines the doublet intensity ratio with the ability of hydroxyl groups in tyrosine to form hydrogen bonds. When tyrosine is "covered", its hydroxyl group becomes a hydrogen donor for hydrogen bonds, and when tyrosine is "exposed", it can be both an acceptor and H donor for the above-mentioned bond. In a case of the non-irradiated collagen film, the band at 855 cm^{-1} has a higher intensity indicating that the aromatic amino acid is "covered" or else that its hydroxyl group is a donor for a hydrogen bond [22].

As a result of irradiation, the intensity ratio becomes reversed suggesting that tyrosine is "exposed" and could be a donor as well as an acceptor for hydrogen bonding. The studied range of 876–855 cm^{-1} can also be interpreted as a disturbance in the amino acid sequence of collagen. These disturbances most likely concern hydroxyproline (855 cm^{-1}), which is an imino acid occurring almost exclusively in the collagen chain and plays a significant role in maintaining of the helical structure [30,9–11]. Hydroxyproline forms hydrogen "bridges" and also effectively blocks rotation of the collagen chain stabilizing the triple-helical structure [4]. The stability of the collagen helix depends to a large extent on the percentage content of proline and hydroxyproline. If the difference in peak profile observed in the Raman spectra is due to hydroxyproline, then this is an indication of a transition from an ordered to a random coil structure. The band at 1004 cm^{-1} assigned to phenylalanine remains practically unchanged.

Comparing the non-irradiated spectra with irradiated ones of collagen films using a single pulse of UV light we observed that the band at 815 cm^{-1} was changed significantly (change of shape and "smoothing"). This band is assigned to the stretching vibration C–C in the main chain of the collagen molecule. Such a large change suggests the possibility of these bonds breaking under the influence of the incident laser pulse. It is probable because the photon energy generated by KrF excimer laser is relatively high and equals 5.0 eV. The dissociation energy of C–C bonds is lower (3.6 eV only), thus the energy in the order of 5.0 eV is sufficient to cause direct bond breaking [4,31] within the main chain of the collagen molecule.

In our recent works we have considered the formation mechanism of the "micro-foam" structure on the collagen film surfaces in the photomechanical regime. The above-mentioned structure is a result of laser pulses but it is not a classical ablation process leading to the clean surface etching. We can suppose that the changes in the internal structure of the collagen molecule are also due to the photochemical pathway bringing about a decomposition of the main chain [32].

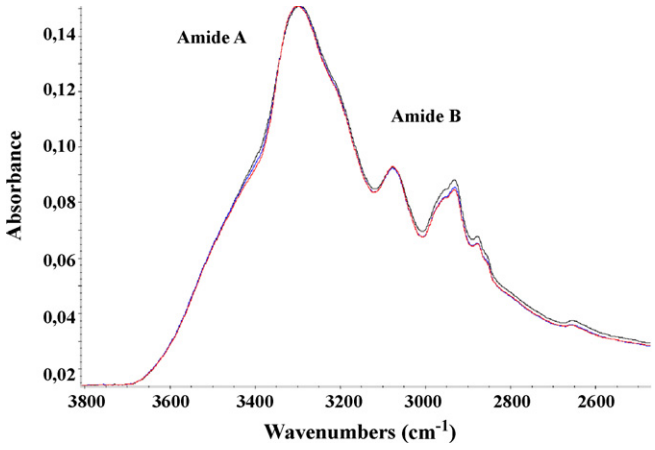


Fig. 2. FTIR-ATR (3800–2700 cm^{-1}) spectra for collagen film surface: non-irradiated (red line) and exposured to single laser pulses at a fluence of 0.4 J/cm^2 (blue line-3 pulses, black line-4 pulses).

It is known that the other processes can compete with the photochemical dissociation [4,33,34]. It is generally agreed that the energy of nanosecond laser pulses is initially transformed into electronic excitation but subsequent energy transfer can take a variety of forms (the internal conversion of absorbed photon energy to the vibrational modes of the molecule). It is the basis of the photothermal mechanism of the ablation process [4]. In practice, distinction between these mechanisms is difficult because of limited experimental data.

3.2. FTIR-ATR spectra

In Figs. 2–5 we present FTIR-ATR spectra of collagen films before and after irradiation with single laser pulses with energies of 0.4–1.7 J/cm^2. The main bands seen in the recorded spectra are located at the following wavenumbers: 3300, 3078, 1629 and 1543 cm^{-1}. The broad band at 3300 cm^{-1}, amide A, is due to the Fermi resonance of N–H stretching and the overtone of amide II [21,23,34]. The band at 3078 cm^{-1} is assigned to amide B whereas the wavenumbers of 1629 and 1543 cm^{-1} are

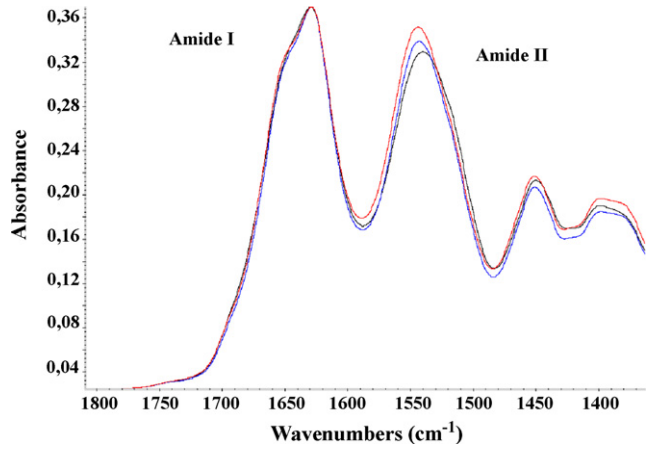


Fig. 3. FTIR-ATR (1800–1400 cm^{-1}) spectra for collagen film surface: non-irradiated (red line) and exposured to single laser pulses at a fluence of 0.4 J/cm^2 (blue line-3 pulses, black line-4 pulses).

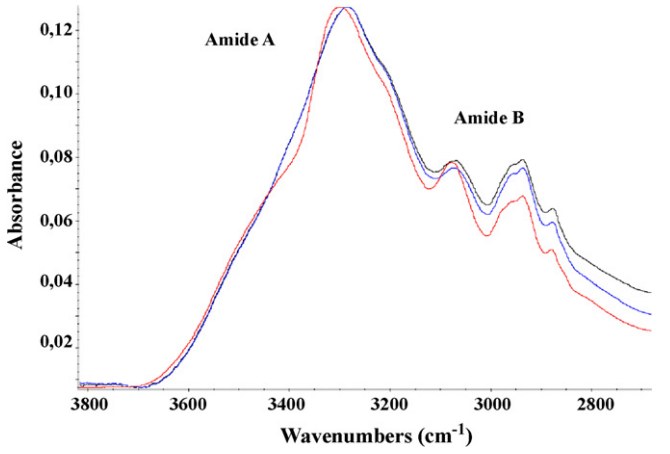


Fig. 4. FTIR-ATR (3800–2700 cm^{-1}) spectra for collagen film surface: non-irradiated (red line) and exposured to single laser pulses at a fluence of 1.4 J/cm^2 (black line-1 pulse, blue line-2 pulses).

related to the maximums of amide I (about 80% C=O stretch) and II (40% C–N stretch, 60% N–H bend) bands [21]. All above bands are very complex and respond to both vibrations assigned to water molecules and vibrations, which are an indication of the contribution of the structures: triple helix and random coil [21,23,35,36]. Thus, the spectral changes as a result of laser irradiation can be explained as conformational distortions taking place in the collagen molecule. It is also noteworthy that the collagen spectrum for the sake of specific amino acid sequences (proline and hydroxyproline presence) and the ability of N–H group to form hydrogen bonds has several characteristic features, which differentiate them from the typical spectra of other proteins. It concerns the amide A band (stretching vibrations of N–H group) which in the collagen spectrum usually appears at 3325–3330 cm^{-1}. These frequencies are 25 cm^{-1} higher at least in comparison with the spectra of other proteins. The stretching vibrations of the N–H group occur usually in the range of 3400–3440 cm^{-1}. As a result of the formation of a hydrogen bond network by the above-mentioned group, the band position is shifted to the lower wavenumbers (around 3300 cm^{-1}) [21].

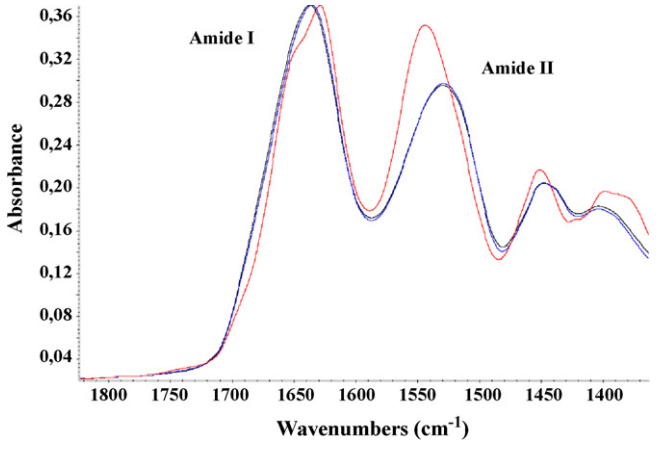


Fig. 5. FTIR-ATR (1800–1400 cm^{-1}) spectra for collagen film surface: non-irradiated (red line) and exposured to single laser pulses at a fluence of 1.4 J/cm^2 (black line-1 pulse, blue line-2 pulses).

Table 2
Amide bands position in collagen film before and after UV laser irradiation

Fluence (J/cm^2)	Amide band	Bands position (cm^{-1})		
		0 pulse	1 pulse	2 pulses
1.4	A	3300	3285	3285
	B	3078	3074	3073
	I	1629	1635	1637
	II	1543	1529	1530
Fluence (J/cm^2)	Amide band	Bands position (cm^{-1})		
		0 pulse	3 pulses	4 pulses
0.4	A	3300	3298	3295
	B	3078	3077	3076
	I	1629	1629	1630
	II	1543	1543	1540

Distinct from other proteins the peptide bond in the collagen molecule is partially involved in the five-membered ring of proline. In consequence, amide I band (stretching vibration of C=O group) being a part of groups including five-membered rings of the lactame-type is shifted to the higher wavenumbers. Thus, amide I band is usually somewhat broad in comparison with spectra of other protein. It may be due to the overlapping of the amide I band with the band of the proline ring [34].

In Figs. 2 and 3 FTIR-ATR spectra obtained for collagen films non- and irradiated ones using single pulses of UV light at a fluence near threshold value (0.4 J/cm^2) are shown. Irradiation with 3 or 4 laser pulses brought about a small shift of amide A and II bands to the lower wavenumbers. We did not observe any changes within the amide I band. The shapes of above-mentioned bands were also unchanged. More visible changes appeared after 5 pulses suggesting the possibility of water loss by the collagen molecule and thereby inducing disturbances in the internal network of hydrogen bonds. The bands positions, shifts and experimental conditions are summarized in Table 2. For comparison, in Figs. 4 and 5 we present spectra of the collagen "micro-foam" structure obtained at a fluence of 1.4 J/cm^2 (significantly above the threshold) with 1 pulse only. In this case a single pulse was sufficient to shift all amide bands and to cause changes in their shapes. The amide A, B and II bands were shifted to the lower wavenumbers and amide I band to the higher wavenumbers. These changes point to breaking of intermolecular hydrogen bonds formed either directly between α-chains or through water molecules [11].

Water plays a significant role in the triple helix structure because it is involved in maintaining the conformation of the collagen molecule and it influences the physical properties of collagen fibrils as well [37]. As we wrote before, hydrogen bonds in the triple helix structure arise between glycine amine group of the single helix and the carbonyl group in the X position according to the scheme known as Rich and Crick model II [38]. Further stabilization of this structure is due to the presence of water, which is most probably bound by the "water bridges" marked as α, β, γ and δ. The α- and γ-bridges occur in the single chains and they are formed between two carbonyl groups of proline and glycine, respectively, and between the hydroxyl

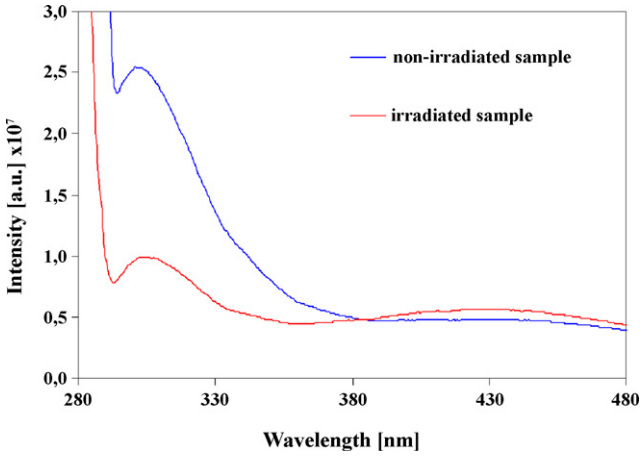


Fig. 6. Fluorescence spectra for collagen film sample in the range of 280–480 nm: non-irradiated (blue line) and irradiated using 1 pulse at a fluence of 1.0 J/cm^2 (red line). The excitation wavelength was λ_{ex} = 270 nm.

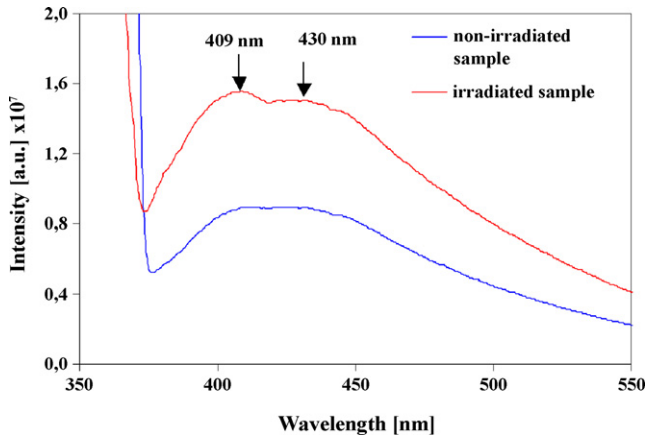


Fig. 7. Fluorescence spectra for collagen film sample in the range of 350–550 nm: non-irradiated (blue line) and irradiated using 1 pulse at a fluence of 1.0 J/cm^2 (red line). The excitation wavelength was λ_{ex} = 350 nm.

group of hydroxyproline and carbonyl groups of glycine. The β-(through three or four water molecules) and δ-bridges (through two or three water molecules) can combine carbonyl groups of glycine and proline in the adjacent chains and the hydroxyl groups of hydroxyprolines with carbonyl groups of prolines in the adjacent α-helixes [9–11]. The water loss in KrF laser irradiated collagen films (bound both in the single chain and between adjacent chains), and reduction of hydrogen bonds stabilizing the triple helix structure resulting from this process, might be the cause of changes in the amide band positions observed in the FTIR-ATR spectra. It should be emphasized that a single laser pulse at a fluence of 1.4 J/cm^2 is quite sufficient to cause a maximum shift of the bands leading to the transition of the ordered structure to a random coil structure. Next pulses do not influence significantly the shape and amide bands position suggesting that all processes take place during irradiation using 1 pulse only. The FTIR-ATR results seem to be in good agreement with results obtained by Raman spectroscopy.

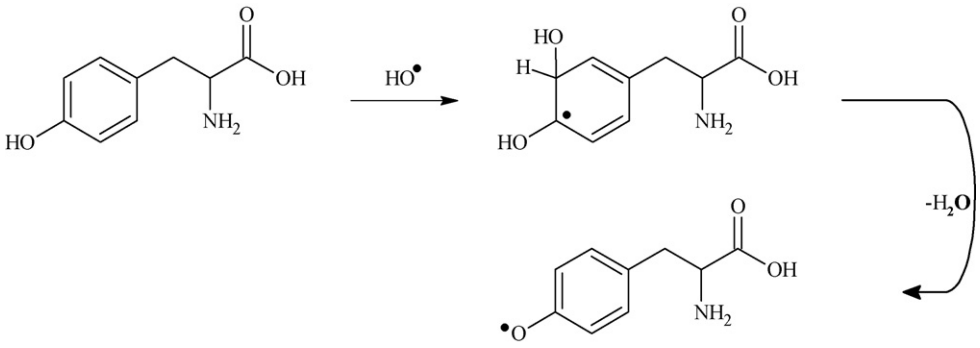
The alternative explanation of the changes occurring within amide I band (shape change and its sharpening, Fig. 5) is photochemical decomposition of the five-membered ring in proline [34]. The photons energy in the order of 5.0 eV generated by KrF excimer laser might be considered to cause a partial destruction of the lactame-type ring of the proline which is also involved in peptide bond formation. It is common knowledge that the imino acid-proline, influences essentially the stabilization of the ordered structure. If we assume that photochemical decomposition takes place, all interaction by means of hydrogen bonds and "water bridges" within or between α-chains will undergo destruction as well.

3.3. Fluorescence spectra

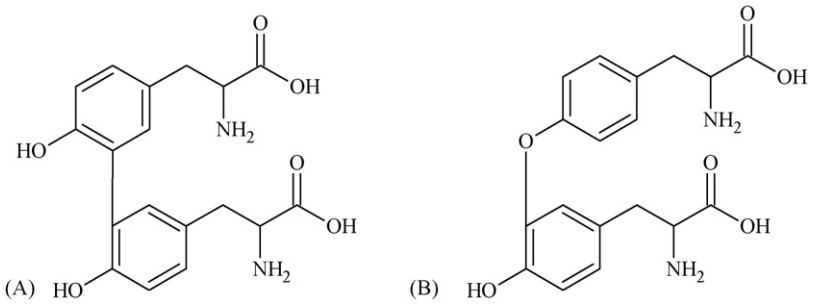
In Figs. 6 and 7 we present fluorescence spectra of collagen films before and after laser irradiation with a single pulse at a fluence of 1.0 J/cm^2. Two excitation wavelengths, 270 and 350 nm, were applied.

Under excitation at 270 nm the fluorescence spectrum (Fig. 6) of the sample shows a well-pronounced maximum at 302 nm in the case of the non-irradiated area of the film and at 303 nm for the laser irradiated spot, a similar wavelength with a comparatively significant reduction of intensity. The broad band at 303 nm is usually assigned to tyrosine, which due to the presence of an aromatic ring is a main chromophore capable of absorbing UV radiation ($\pi \rightarrow \pi^*$ transition) [4]. Though other aromatic amino acids in the collagen polypeptide chain (phenylalanine) can be excited in the same range at 270–290 nm, it is the tyrosine with a quantum yield of fluorescence being ~0.14 (for Phe ~0.04), which is responsible for the above-mentioned process taking place in the collagen film [3,39]. The spectra analysis showed that the band intensity at 303 nm (irradiated area) decreased under the influence of the incident laser pulse rather indicating a substantial disappearance of the chromophoric tyrosine molecule (Fig. 6). At the same time, along with decreasing band intensity at 303 nm we observed an emission increase in the range of 375–550 nm (Figs. 6 and 7). The control sample (non-irradiated area of the collagen film) does not reveal significant fluorescence in this range applying excitation wavelength 270 nm, but as a result of laser irradiation we can notice its gradual increase. This is well seen also by using the excitation wavelength 350 nm (Fig. 7) which increases further its band intensity. This is pointing out the formation of at least two new fluorophores since we can distinguish two areas in this band with emission maxima at 409 and about 430 nm. They may be assigned to the photo-oxidation products of tyrosine: di-tyrosine and 3,4-dihydroxyphenylalanine (DOPA), respectively, with related products [3,29,39–42].

It is known that photo-oxidation of proteins including collagen can occur via two major pathways. The first of these involves direct photo-oxidation arising from the absorption of UV radiation by the protein, or bound chromophore groups generating excited states (singlet or triplets) or radicals by photo-ionisation (Type I). The second process is due to indirect oxidation of the protein via the formation and subsequent reactions of singlet oxygen generated by the energy transfer to the ground state



Scheme 1.



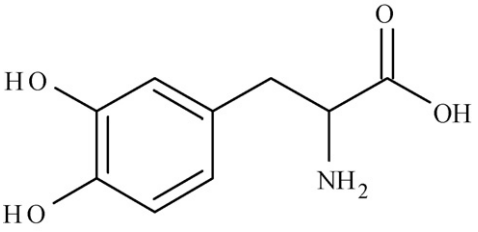
Scheme 2.

molecular oxygen (triplet) by either protein-bound, or chromophores (Type II) [29,39,41,42].

Tyrosine residues absorbing UV laser light, under the influence of various oxidation agents are transformed into phenoxyl radicals. In our previous papers we have shown that collagen Type I from rat tail tendon contains only small amount of tyrosine [43].

However, flash photolysis experiment showed that the transient spectra of collagen solution excited at 266 nm show two bands. One of them with maximum at 295 nm and the second one with maximum at 400 nm. The peak at 400 nm is assigned to phenoxyl radicals of tyrosine [44]. This process can occur either by direct oxidation of the aromatic ring of the amino acid with hydroxyl group deprotonation or as a result of addition–elimination reactions (Scheme 1) [42]. Two phenoxyl radicals may undergo a self-dimerisation process resulting in the formation of di-tyrosine products: carbon–carbon dimer (A) and carbon–oxygen dimer (B) (Scheme 2) [3,40,41,45].

Reaction between hydroxyl radical and aromatic side chain of tyrosine leads to the formation of new radicals which may undergo further alterations giving 3,4-dihydroxyphenylalanine (DOPA) (Scheme 3) [3,41,46,47]. DOPA formation in the presence of oxygen takes place by rapid elimination of peroxide radical [41].



Scheme 3.

4. Conclusions

Collagen films exposed to single pulses of KrF laser irradiation undergo the surface "foaming" giving rise to the formation of the "micro-foam" structure having interesting biomimetic properties.

Raman and FTIR-ATR spectra analysis showed that UV laser light is capable of inducing conformational changes in the irradiated collagen films, mainly as a result of breaking of the hydrogen bond network and loss of water molecules which maintain the ordered structure. Partial decomposition of the collagen main chain is also a possibility, but in any case it is limited to a minimum due to the mechanism which is essentially a low temperature tension wave assisted boiling phenomenon.

Fluorescence measurements showed that UV light generated by the KrF excimer laser is capable of causing tyrosine oxidation by generating excited states or radical photo-ionisation. As a result of UV absorption the products of tyrosine photodegradation: di-tyrosine and DOPA might also be considered.

The obtained results indicate that the interaction between the collagen film and UV laser radiation discussed in this work might be considered not only in the photomechanical regime as we presented in our recent works [14–17], but also from the photochemical mechanism point of view with reactions of electronic excited states.

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