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Mechanisms of the Influence of UV Irradiation on Collagen and Collagen-Ascorbic Acid Solutions

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The study of the influence of UV irradiation on collagen solutions has shown the destabilization of the collagen molecule by calorimetric method. It is reflected both in changes of thermodynamic parameters of transition (Tm, ΔH, $Cp = f(T)$) and in the appearance of a low temperature peak, that is practically irreversible against rescanning. All these indicate that the important defects in the molecule occur. The ESR measurements have shown that the above-mentioned thermal changes are connected with the occurrence of free radicals in solution under UV irradiation. They interact with proline (Pro) residues of the protein with the appearance of secondary free radicals, with following migration to glycine (Gly) residues. The emergence of the free radicals at the Pro and then at the Gly residues may cause the dramatic structural defect resulting from the UV irradiation, which significantly alters the network of hydrogen bonds in the triple helix of the collagen molecule. All this is connected with destabilization of the collagen molecule, because the defects in amino acid residues probably lead to cleavage of covalent bonds near the damaged sites maintaining the triple helical structure. The presence of ascorbic acid in collagen solution protects the collagen molecule from occurring of secondary free radicals.

1. INTRODUCTION

Collagen represents the most abundant animal protein and performs the function of the main structural protein as well. Therefore, the disorder in the biosynthesis or in protein structure as a result of the influence of some physicochemical factors causes significant changes in the steadiness of organism (the functional destabilization of skin, bone, and tendon molecular ensembles). UV irradiation presents one of the important external factors evoking the destabilization of collagen molecule that is a precondition of skin aging (the biological aging follows it) and wrinkling. That is why the study of UV irradiation on the collagen macromolecule is significant both for fundamental and applied biophysics and for medical biophysics. There are some interesting studies and views [1–6] referring to this problem, but molecular mechanisms for the influence of UV light on collagen are still unknown. Many studies have demonstrated modification of collagen evoked by UV radiation: it has been shown that in solution, collagen loses the ability to form natural fibrils after irradiation [6]. Moreover, photocrosslinking and photodegradation of collagen may also occur on exposure to UV radiation [1–6].

The investigation of the photochemical properties of collagen Type I in acetic acid solution was also carried out using nanosecond laser irradiation [7]. The transient spectra of collagen solution excited at 266 nm showed a peak of tyrosyl radicals at 400 nm.

The reactions of hydrated electrons and $O\dot{H}$ radicals with collagen have been studied by pulse radiolysis. In the absorption spectra of products the tyrosine radicals were found as well resulting from the reaction of the hydroxyl radicals with collagen [7].

Many researchers [8–14] have investigated the influence of UV radiation on collagen in films. It was found that after UV irradiation of thin collagen films random-coil domains increased on the surface [10, 12].

It is known that using different protective systems may prevent damages, which appeared as a result of irradiation. Modifications of photochemical stability of collagen in the presence of β-carotene [15, 16], riboflavin [17], melanin [18], methylene blue [19], H_2O_2 and thiourea [6] were reported. Ascorbic acid represents also one of such systems against the appearance of free radicals.

The purpose of this work is to study the influence of ascorbic acid on photochemical transformation in collagen in acetic acid solutions.

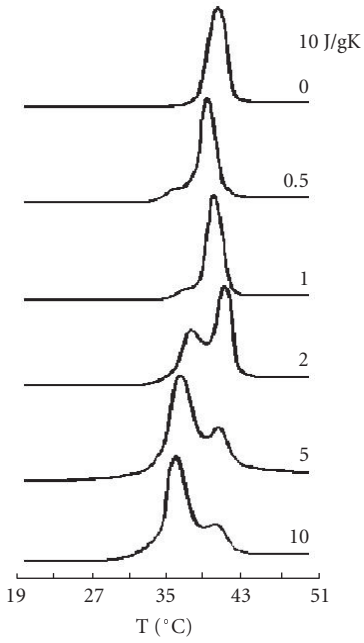


Figure 1: Heat denaturation peaks of irradiated collagen: the numbers in the picture indicate duration of the irradiation in minutes. In each experiment protein concentration in solution is 0.6 mg/ml, pH 3.7.

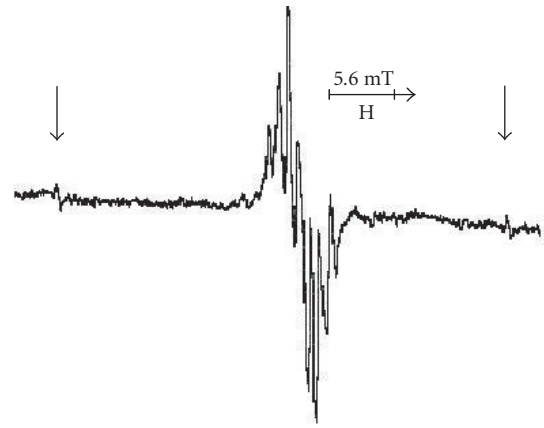


Figure 2: The ESR spectrum with seven superfine components (ΔH = 1.13 mT, g-factor 2.001) of irradiated collagen (77 K; pH 3.7).

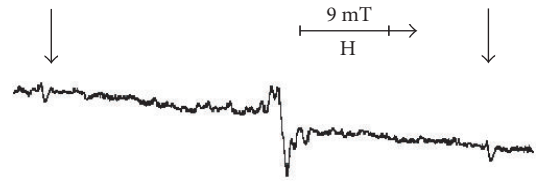


Figure 3: The ESR spectrum with five superfine components (ΔH = 1.16 mT, g-factor 2.003) of irradiated acetic acid solution (pH 3.7; 77 K). (The arrows indicate a doublet ($\Delta H \sim 50.6$ mT) of hydrogen atom.)

2. EXPERIMENTAL DETAILS

The rat tail tendon collagen has been used as a subject of investigation. The preparation and purification of protein were implemented by the method of Glimcher [20]. The sample was studied as a diluted solution (protein concentration −0.6 mg/ml); acetic acid was applied as a solvent (pH 3.7). The mercury-quartz irradiator (mercury lamp DRT 230) was used as a source of irradiation. It gives a continuous spectrum of radiation in the whole UV region. The intensity of irradiation was 1.8 J/cm^{2}min. The intensity of the incident irradiation was measured using a radiometer IMO-2N (Russia). All experiments were conducted in the same irradiation conditions. The thermodynamic measurements were carried out using microcalorimeter DASM-4 (Pushino, Russia). For ESR experiments, collagen solution droplets were frozen in liquid nitrogen; the droplets were collected in a quartz vessel. The ESR spectra were recorded with the ESR-V radiospectrometer. The powder of Mn(II) in MgO served as the ESR standard.

3. RESULTS AND DISCUSSION

The calorimetric measurements have shown the "destructive" influence of UV irradiation on the collagen molecule, which is reflected both in changes of thermodynamic parameters of transition (Tm, ΔH, $Cp = f(T)$) and in the appearance of a low temperature peak, which rises with increasing irradiation dosage (see Figure 1) [21]. The heat absorption redistribution between the peaks takes place. The above-mentioned facts prove that the influence of UV irradiation causes the damage in the structure of the collagen molecule. Miles and coauthors [6] suggest that by the influence of UV irradiation the "coil-random coil" transition in collagen goes via the intermediate state. That is triple helical, but there are some damages in all α-helixes, caused by the appearance of free radicals in the solution.

As a result of the collagen solution irradiation at a temperature of 77 K, the ESR signal with the seven superfine components of ΔH = 1.13 mT and g = 2.001 occurs. During the UV irradiation of the collagen solution, besides the seven components, the ESR signal contains a doublet with a split of $\sim$ 50.6 mT, which is due to the hydrogen atom (see Figure 2). A similar doublet together with an acetic acid radical appears in the ESR spectrum of irradiated acetic acid aqueous solution (pH = 3.7) at 77 K (see Figure 3). The ESR measurements have shown that in the acidic medium an emergence of the hydrogen atom occurs:

$$e + H^+ \longrightarrow \dot{H} \quad (1)$$

which is unstable and easily goes into the radical reactions:

$$RH + \dot{H} \longrightarrow \dot{R} + H_2. \quad (2)$$

In the presence of collagen molecules in solution, all the

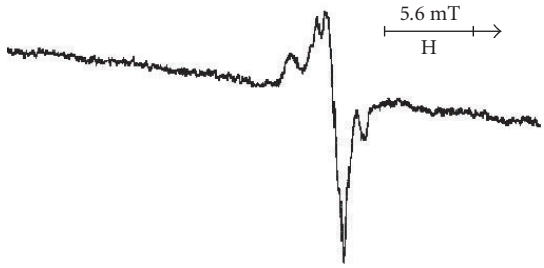
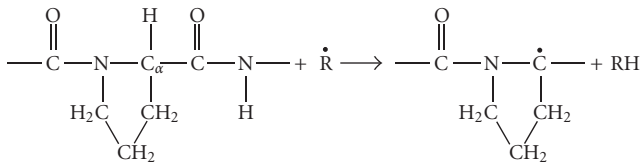


Figure 4: The ESR spectrum with three superfine components (ΔH = 3.11 mT, g-factor 2.003) of irradiated collagen (223 K, pH 3.7).

above free radicals may interact with a macromolecule, according to the general scheme:

$$\mathrm{H - COl + \dot{R} \longrightarrow C\dot{O}l + RH.} \quad (3)$$

Probably, the site for the radical appearance in the collagen molecule is the Pro residue, because in this case only the interaction of nonpaired electron with six protons happens, which determines the emergence of seven superfine components in the following ESR spectrum:



The Ṙ-radical interacts with the hydrogen of the C_α-carbon. The free electron appearing on carbon is delocalized at the π-bonds of the indole ring, as a result of which it equally interacts with the six protons of CH_2 groups.

In the case of sample heating up to 223 K in the ESR spectrum instead of seven superfine components, the triplet is observed (see Figure 4). The latter disappears when the sample temperature reaches the 273 K. Such a change of ESR spectrum can be explained with the migration of free radical from the Pro residue to Gly, because the triplet spectrum reflects the interaction of unpaired electron with two protons.

It is known that free radicals contribute to the normal organism functioning and the developing of some pathological processes. The damages induced by the influence of UV irradiation may be avoided due to some protective systems, such as ascorbic acid. Besides its multifunctionality, ascorbic acid takes part in oxidation-reduction reactions as in biosynthesis of collagen. That is why the influence of the UV irradiation on collagen in the presence of ascorbic acid has been studied.

The calorimetric study has shown (see Figure 5) the gradual decrease of irradiated collagen molecule destabilization that is reached by adding of ascorbic acid to the solution [22].

The singlet spectrum of the collagen solution in the presence of ascorbic acid (width 1.74 mT, g-factor 2.0047) is shown in Figure 6. On the one hand, the spectrum with seven superfine components (see Figure 2), and on the other hand,

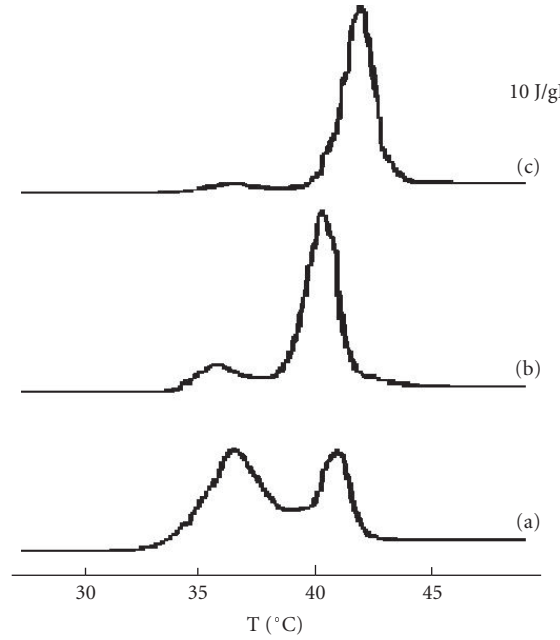


Figure 5: The melting curves of irradiated collagen in the presence of ascorbic acid, the ascorbic acid concentration is (a) 0.6, (b) 0.8, (c) 2.4 mg/ml, accordingly (the collagen concentration in solution is 0.35 mg/ml. Duration of the irradiation is 5 min).

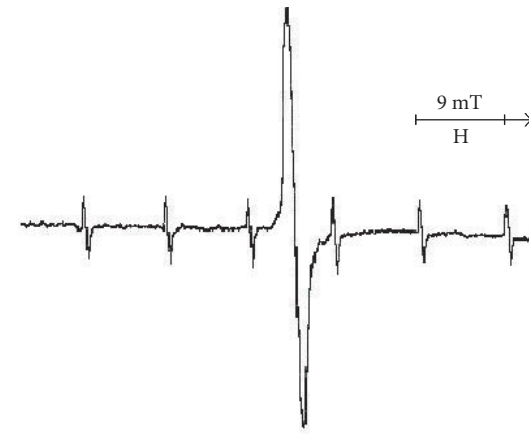


Figure 6: The ESR singlet spectrum (ΔH = 1.74 mT, g-factor 2.0047) of irradiated collagen in the presence of ascorbic acid (77 K, pH 3.7).

the future of ascorbic acid takes part in radical reactions to neutralize the reactivity of free radicals, the real action of ascorbic acid against free radicals becomes clear.

4. CONCLUSION

The UV irradiation causes the appearance of primer free radicals (acetic acid radicals and hydrogen atoms) in the surrounding water, which affect with Pro residues in the collagen molecule appearing as secondary radicals. It may cause

the weakening and then cleaving of covalent bonds near the damaged residues in the single chain maintaining the triple helical structure. The presence of ascorbic acid in solution protects the collagen molecule from the appearance of the secondary free radicals.

ACKNOWLEDGMENT

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