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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.