FIBRIL FORMATION OF COLLAGEN *IN VITRO* AT TEMPERATURES CLOSE TO PHYSIOLOGICAL

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Collagen of type I forms part of fibrils and fibers of most connective tissues. The structure of collagen fibrils of the skin and internal organs (the heart, lungs, liver, kidney) is composed of complexes of type I collagen with collagens of types III and V. Fibrils in the skin of an adult organism that possess both rigidity and flexibility have a relatively small diameter (about 60 nm). In the skin derma, collagen fibrils form a mobile and flexible network, which fulfils the mechanical function and is involved in the functioning of cells. The structure of collagen fibrils is determined not only by the diversity of types of macromolecules entering into their composition but also the conditions of fibrillogenesis. Temperature affects the conformation of collagen molecules and thus the self-assembly of collagen into fibrils [1]. The formation of native collagen fibrils begins after the microunfolding of local regions of the triple helix, which have a low stability [2, 3]. It follows from the literature data that the unfolding of labile regions at the ends of the triple helix of collagen from the skin of animals occurs in the temperature range of 28–41°C [4–6]. Studying the formation of fibrils *in vitro* by the action of temperature gives an insight into the molecular mechanisms of the collagen fibril formation *in vivo*.

The goal of the present work was to study the formation of collagen fibrils at temperatures of the close physiological. We developed a system of fibrillogenesis, which consisted of collagen from the skin of two-month-old pigs and phosphate-buffered saline (PBS), pH 7.0, I = 0.145 M. The fibril formation was recorded from changes in the optical density (D) of collagen solutions in thermostated quartz cuvettes on a Specord UV VIS spectrophotometer (Karl Zeiss, Germany) at a wavelength of 400 nm. The time of fibril formation was defined as the time the curve of changes in optical density reaches a plateau.

For the electron microscope examination, pieces of collagen gel were fixed for 2 h in a solution containing 2.5% glutaraldehyde and 3% paraformaldehyde in Na cacodylate buffer. Slices were obtained from blocks of high hardness on a Leica EM UC6 microtome using a diamond cutter and placed on carbon-fixed support films, contrasted with uranyl acetate and lead citrate, and analyzed on a JEOL 1200EX microscope. The diameter of fibrils was measured at the centre of their longitudinal section on negatives scanned with a resolution of 2400 dpi using the program IGL Trace 1.26b.

The study of the kinetics of collagen fibril formation depending on temperature shows that, as temperature increases from 28.5 to 38.5°C, the rate of fibril formation and the time of the formation of the stable structure of

fibrils increases two- to threefold. The optical density of the collagen gel in the plateau phase decreases, which may indicate changes in the number of fibrils and/or their sizes. The results of the electron microscope examination of the structure of collagen fibrils formed at different temperatures show that the diameter of fibrils is the greatest (58–68 nm) at T = 28.5–32.5°C. An increase in temperature to 34.5°C leads to a decrease in the diameter to 41 nm. As temperature to increases to 36.5°C, the diameter of fibrils decreases to 28 nm. We showed that, by creating temperature conditions close to physiological, it is possible to significantly affect the diameter of fibrils, which fulfils an important functional role. The number of fibrils of certain diameter also depends on the temperature of their formation. Fibrils of minimal diameter, obtained at 36.5°C, compile more homogeneous population of fibrils as compared with the fibrils formed at temperatures lower (34.5°C) and higher (38.5°C) by two degrees than physiological. At T = 28.5–32.5°C, an inhomogeneous population of fibrils is formed.

Thus, we found that an optimal temperature for the formation of fibrils from this collagen is 36.5°C. Physiological temperature stimulates a strong intermolecular binding of collagen molecules from the pig skin into fibrils. We found conditions for the formation of homogenous fibrils of a minimal diameter (about 28 nm): PBS, pH 7.0, I = 0.145 M, T = 36.5°C, C = 1.5 mg/ml.

Collagen fibrils reconstructed in the present work can find application in cell and tissue engineering. On the basis of collagen fibrils from the pig skin, it is possible to create analogues of various connective tissues: skin derma, heart valves, and blood vessels.

References

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PIRIMILPHOS-METHYL EFFECT ON SKELETAL MUSCLE CONTRACTION DYNAMICS

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Pesticides are an permanent ecology factor. Environment saturation with potentially dangerous substances cause numerous pathologies. Organophosphorus pesticides as a high-performance agent against broad variety of insects and low persistence substance are widely used in nowaday agronomi-