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Метод дослідження властивостей колагеноподібних пептидів, що застосовуються в сучасних медичних технологіях

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Method for the Study of Properties of Collagen-Type Peptides Used in Modern Medical Technology

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Пропонується метод дослідження кінетики конформаційних перетворень в колагеноподібних структурах. В сучасній медицині зазначені структури використовуються для отримання інформації щодо характеру розповсюдження злоякісних пухлин та особливості їх морфологій при взаємодії з фармацевтичними препаратами.. Для отримання зазначеного параметру запропоновано експериментальну методику.

Ключові слова: кінетики переходу «клубок-спіраль», колагеноподібні структури, розсіяння світла

A method for study of nonlinear kinetics of coil-helix transition in collagen-type structures has been proposed. The method has been tested for one of such structures with molecules having 40 nm in length. The proposed physical method is based on the fact that as a result of coil-helix transition collagen-like particles change their shape On the basis of experimental data a time of shaping of triple helix has been estimated. Thus, the experimental method provides for the study of light scattering in liquid system «water + denatured collagen». The given method has been proposed to be used in selecting a length of CMP (*Collagen Mimetic Peptides) molecules used in the modern medical science.*

Key Words: kinetics of coil-helix transition, collagen-type structures, light –scattering

Статтю представив академік НАН України, д.ф.-м.н., проф. Булавін Л.А.

Introduction

A lot of researches have dealt with mechanisms of coil-helix transition in collagen-type structures [see, for example 1, 2]. However, as it has been stated in research [3], many kinetic and theoretical problems of coil-helix transition have still been unsolved [4,5]

It is known [6] that collagen is the main structural albumen of the connective tissue that ensures stability of its skeleton. In addition, collagen is one of the most biologically stable and compatible natural polymers used for the development of synthetic biological materials and theraupetic methods [7]. Physical methods are of paramount importance for determining a structure of bio-molecules [8]. This research deals with experimental method to investigate nonlinear kinetics of helix-coil transition in collagen-like structures and possible ways of its application in the modern medical science.

Method

The proposed physical method is based on the fact that as a result of coil-helix transition collagen-like particles change their shape [9]. In connection therewith, two well-known approaches have been used: selection of a model system, the aqueous solution of denatured collagen [10] and application of molecular light scattering method in the course of experiment [3].

Thus, the experimental method provides for the study of light scattering in liquid system «water + denatured collagen». The collagen concentration has been (0.05; 0.1; and 0.2) g/l. Light scattering has been measured at a scattering angle of 45º. The use of this method is caused as follows:

Aqueous solution of denatured collagen (gelatin) is known to be a disperse system at a temperature of 70^0C, with separate peptide chains playing a role of the disperse particles. As temperature decreases, at a concentration of dissolved matter less than 0.4 g/l, these disperse particles are shaping into individual aggregates. These aggregates have triple-helix collagen-like structure [11]**.** Shaping of these aggregates is caused by changes in conformations of peptide chains, as a result of coil-helix transition

According to the classic theory of molecular light scattering [12] such structural transformations lead to an increase in intensity of light scattering by disperse medium. The study has been carried out with the use

of installation described in [13]. The scheme is given on Figure.1, where 1 is a lamp; 2, 11 are light filters; 3 is a glass plate dividing light into two beams; 4 is a cuvet with solution of investigated substance; 5 is a light trap; 6 is a glass diffuser; 7, 7', 9, 9' are lenses; 8, 8' are compensating septa; 10, 10' are rhombic prisms; and 12 is an ocular.

The experimental curves are showed on Figure 2, where ξ is a ratio of scattering intensity at any time $I(t)$ to intensity of scattering at the initial time I_0. The initial time is considered be a time of preparation of the solution.

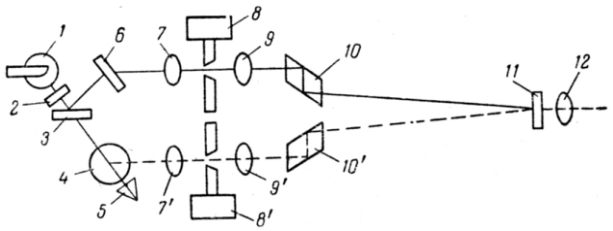

Fig.1. Experimental installation

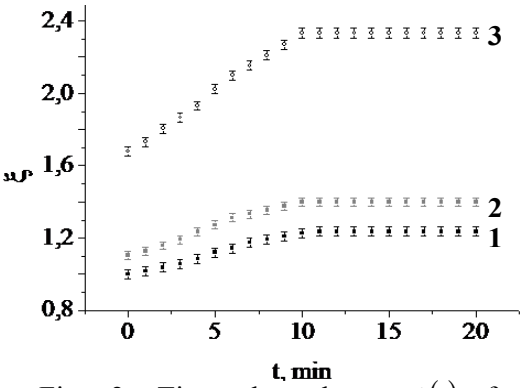

Fig. 2. Time dependence $\xi(t)$ for gelatin solution: concentration 0.05 g/l (1); 0.1 g/l (2); and 0.2 g/l (3).

It is known [14] that at temperature 40ºC liquid system «denatured collagen + water» is transformed into gel with chains shaping a grid. It is also known that before this transition there is a stage when the coils are reshaping into helices. In order to process the experimental data the classic theory of light scattering in solutions has been used. According to this theory light scattering I is defined by the formula:

$$I = HcMP(\theta), \tag{1}$$

Where c is concentration of solved substance; M is its molecular mass; H is a constant for this system of substance and solvent; $P(\theta)$ is some function of scattering angle depending on properties of scattering particles. General view of this function is as follows:

$$P(\theta) = \frac{1}{2} P_{\upsilon}(\theta)\left(1 + \cos^2\theta\right), \tag{2}$$

Where $P_{\upsilon}(\theta)$ for coil is defined as

$$P^K(\theta) = \frac{2}{x^2}\left(e^{-x} + x - 1\right), \tag{3}$$

And that for helix is

$$P^P(\theta) = \frac{1}{y} Si(2y) - \left(\frac{\sin y}{y}\right)^2. \tag{4}$$

In the above formulas:

$$x = 16\pi^2 \frac{R^2}{\lambda^2} \sin^2\frac{\theta}{2}, \tag{5}$$

$$y = \frac{2\pi L}{\lambda} \sin\frac{\theta}{2}, \tag{6}$$

$$Si(2y) = \int_0^{2y} \frac{\sin t}{t} dt, \tag{7}$$

where R^2 is gyration radius of a coil; L is a helix length; λ is a length of light wave. At the initial time all chains are shaped as coils. In this case intensity of light scattering is determined as:

$$I_0 = HcMP^K(\theta). \tag{8}$$

Later, a portion of coils are reshaping into helices. If c^K and c^P are concentrations of coils and helices, respectively, intensity of light scattering may be expressed as follows:

$$I(t) = Hc^K(t)MP^K(\theta) + Hc^P(t)MP^P(\theta). \tag{9}$$

Having divided formula (9) by formula (8) we obtain:

$$\xi = 1 + \frac{c^P(t)}{c}\left(\frac{P^P(\theta)}{P^K(\theta)} - 1\right). \tag{10}$$

Length of light wave in our experiment was equal to 417 nm, length of denatured collagen was ~ 300 nm. Value of function $P_P(\theta)$ for these figures is equal to 0.74. At $t \to \infty$ concentration of helices approaches c: $c^P(t) \to c$. According to the formula (10):

$$\xi \to \frac{P^P(\theta)}{P^K(\theta)} = a. \tag{11}$$

Condition (11) allows us to calculate $P^K(\theta)$ with the help of experimentally measured asymptotic values of ξ.

Finally, we obtain formula for concentration of helices:

$$c^P = c\left(\frac{\xi - 1}{a - 1}\right). \tag{12}$$

Experiment results and discussion

Figure 3 shows the obtained dependences $c^P(t)$ for concentrations 0.05; 0.1; and 0.2 g/l.The curves allow

us to estimate time of coil – helix transition. According to Fig. 3 for the investigated collagen-like peptides having 40 nm in length this time is about 10 minutes.

The proposed method can be used for the study of mechanisms of interaction of CMP (Collagen Mimetic Peptides) synthetic molecule with collagen matrix. According to [15] CMP synthetic molecule that has a structure similar to the collagen structure is a link between the collagen matrix and an embed nano-particle that allows us to determine a status of connective tissue. Also, pursuant to [15] a process of tying CMP with collagen framework consists of several stages. Firstly, a synthetic molecule with captured nano-particle is brought to the investigated part of the organism. Then, it ties with collagen matrix of «CMP + nano-particle» complex due to imbedding the latter into triple-helix structure of albumen molecule. At the last stage, stay of visualizing nano-particles in the investigated part of the organism comes to an end, as a result of change in the structure of CPM molecule when temperature reaches 37ºC. A pattern of imbedding of CMP molecules in collagen fibers is showed on Figure 4.

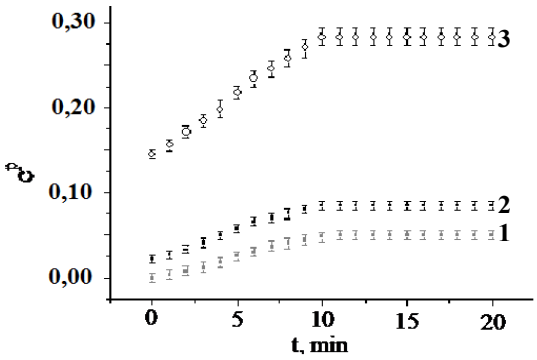

Fig.3. Time dependence $c^P(t)$ for gelatin solution: concentration 0.05 g/l (1); 0.1 g/l (2); and 0.2 g/l (3).

According to [15] it has been established that a change in CMP length influences time of its tie with collagen. As showed in [15] short and long CMP molecules may be used for quick and long investigation, respectively. According to [16], when the triple-helix structure destroys separate peptide chains should arise. It is known that for collagen-like synthetic molecules (for instance, gelatin molecules) destruction of over-molecular structures

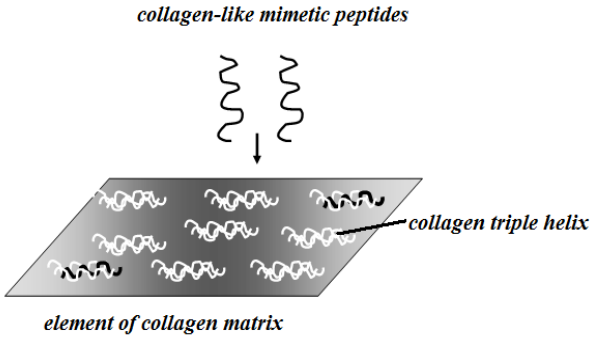

Fig. 4. CMP molecules penetration into collagen matrix

almost coincide with the start of their spatial aggregation [17]. The proposed method is based on this fact. Using the Figures a time of assembly of triple helix may be determined depending on concentration. Proceeding from Figure 3 this time is equal to 10 minutes. Let's assume that time of destruction of triple helix is equal to time of assembly of single helices. Actually, the measured time is a time during which the triple helix exists. As mentioned above, CMP molecules are embedded into triple helix. Consequently, time of existence of triple helix is time when CMP molecule is tied with collagen matrix.

Conclusions

The properties of 40 nm-long synthetic collagen-like molecules structured similar to CMP molecules have been studied with the help of the optic method. Time of assembly of triple helix has been estimated on the basis of the experimental data.

This method is proposed to be used for selecting lengths of CMP molecules. As described above, one of the important parameters influencing effectiveness of the use of CMP molecules in the modern medical science is time when these molecules are tied with collagen matrix. It has been noted also that this time depends on length of CMP molecule. In this study, a method based on measuring intensity of light scattering has been applied to estimate the above time. The method has been tested for collagen-like molecules of 40 nm. The bonding time has been estimated as 10 minutes. This method may be useful for selecting optimal length of CMP molecules when designing nanomedicines.

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