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Вергун Л.Ю.[1] к.ф.-м.н., н.с.

Кінетика денатурації овальбуміну в умовах гіперпереексії: експериментальна методика

[1]Київський національний університет імені Тараса Шевченка, 01601, м. Київ, вул. Володимирська 64/13,
e-mail: vergunliena@rambler.ru

L.Yu. Vergun[1], PhD, Sci. Res.

The kinetics of ovalbumin denaturation at the temperature of hyperpyrexia: the experimental procedure

[1]Taras Shevchenko National University of Kyiv, 01601, Kyiv, Volodymyrska st. 64/13,
e-mail: vergunliena@rambler.ru

Пропонується експериментальний метод дослідження кінетики денатурації глобулярних білків. При застосуванні такого методу визначається період, що характеризується здатністю молекулярної системи до оберненої денатурації. Характерним параметром, який визначає період оберненої денатурації є модуль зсуву. Для апробації методики досліджувалась кінетика овальбуміну в умовах гіперпереексії, що характеризується перевищенням температури 42ºС - граничної межі життєдіяльності живих організмів. В результаті роботи визначений час оберненої денатурації. Зазначений час складає 27 хвилин.

Ключові слова: глобулярні білки, овальбумін, гіперпереексія, модуль зсуву

The new method of studying the denaturation of globular proteins is proposed. Such process the forming of gel is accompanied. The method describes the mechanism of hardeness during gel growing. The torsion pendulum in the proposed method is used. The experimental value in the given method is the shear modulus. The period of the reversible denaturation of globular proteins makes possible to determine in this method. On the example of ovalbumin as the models of globular proteins is established that the period of the reversible denaturation at the temperature of hyperpyrexia (42ºC) composes 27 minutes. The state of the hyperpyrexia is limit of the human viable.

Key Words: globular proteins, ovalbumin, hyperpyrexia, shear modulus

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

Introduction

As is known one of the dangerous physiological states for the human organism is the state, which is characterized by the high temperature of body (more 37,5ºC) [1]. In accordance with the conventional classification of the state of elevated temperature they divide into four groups. For the first group the characteristic value of temperature is within the limits 38–39ºC (low grade), for the second group in the limits 39–40 ºC (moderate), for the third group in the limits 40–41.1 ºC for the fourth group in the limits 41,1 and more degree Celsiuss. Last group is the most dangerous state for the human organism since it approaches the upper limit, compatible with the human life [2].

The danger of this physiological state lies in the fact that in the human organism occur the qualitative disturbances of biological processes [3]. One of the examples of such disturbances is denaturing Human Serum Albumin (HSA) [4]. It begins at a temperature of 42ºC [5]. During this process HSA loses globular native form and converts to the state of coil [6]. The globular protein in the state of the coil cannot fulfill its physiological functions. This form of protein molecule is not compatible with the life. The coil indicated form grid. In the grid the spaces between the chains are filled up with small molecules - water and others: appears structure named gel [7].

As is known for investigating the properties of complex protein molecules (in vitro) are used model systems are proteins with the simpler structure [8]. As a rule during the study mechanical properties of HSA simulate, using small globular proteins (for example ovalbumin). In this case they are based on the similarity of primary structure of three main blast furnaces of these two molecules and on the similarity of their globular structure [9]

It is known that under specific conditions the denaturation can be reversible: the destroyed globular structure can be restored, restoring disturbance in the physiological processes. It is important to determine these conditions, in particular, which period of the reversible denaturatiom is the time interval, with which remains the possibility of the reversible denaturation.

Our purpose is the determination of period of the reversible denaturing of ovalbumin. Solve this

task can, studying the kinetics of the process of denaturation. However, as this follows from the given references, the experimental investigation of kinetics of proteins denaturation in the regime of hyperpyrexia practically were not conducted. The absence of the experimental method, which makes it possible to study this process, is the reason for this. In this article this method is proposed.

Method and materials

As is known in the process of the denaturation of globular proteins is accompanied with the forming of gel. In connection with the fact that stiffness of gel is more than the hardness of protein in the native state, we will name a gel is "rigid phase", leaving after the native protein term is "soft phase". Thus the process of denaturing we will consider as a change in the relationship between the rigid and soft time phase. An increasing of the quantity of rigid phase corresponds to an increasing of the shear modulus of entire system. This circumstance is assumed into the basis of the method proposed: we will study the process of denaturation, controlling an increase in the shear modulus. With the measurements of the shear modulus of globular proteins is necessary to preserve their form in the process of denaturation. The authors propose to solve this problem, after concluding the investigated substance into the tube from another rigid material.

As the experimental apparatus we will use the torsion pendulum. The view of torsion pendulum is represented in Fig.1.

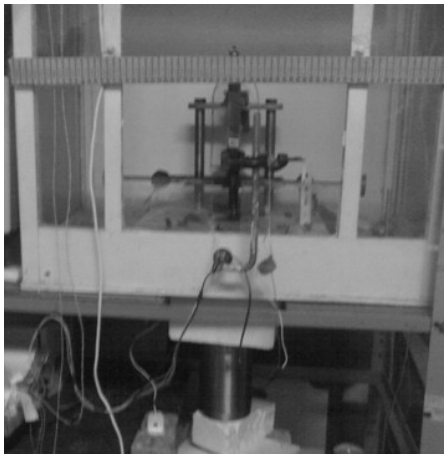


Fig.1. The view of torsion pendulum

The main elements of using device are rod, yoke, metallic thread, clamps with cylindrical cuvette, thermocamera. During measurements the cuvette together investigated subctance is oscillate.

The circuit of the torsion pendulum is represented in Fig.2.

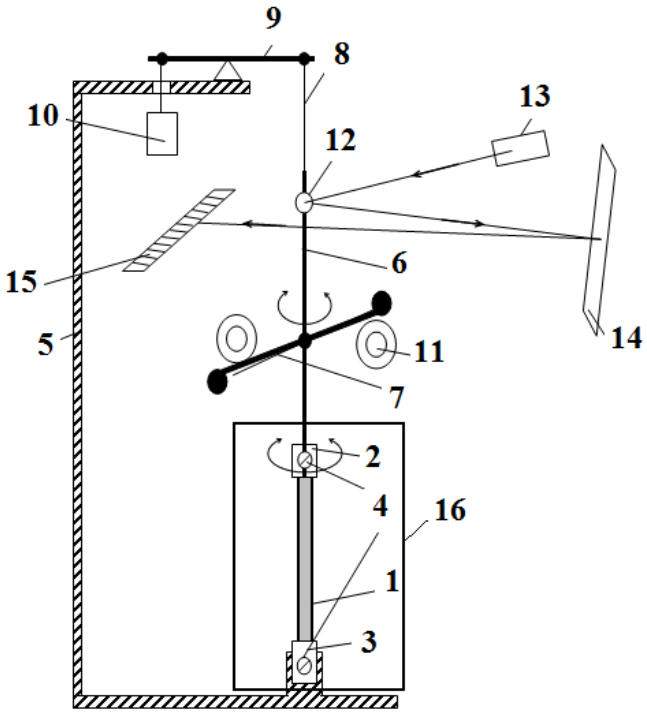
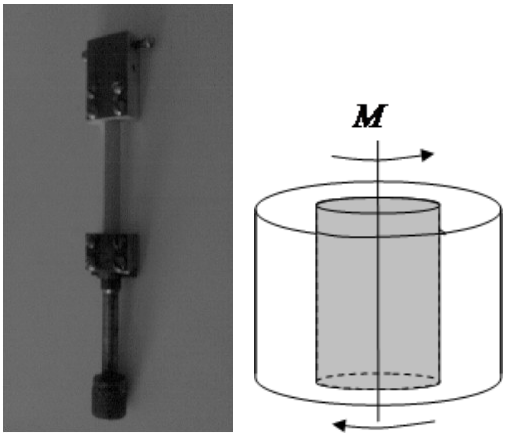


Fig.2.The circuit of the torsion pendulum

In this plan is marked by 1-sample, 2,3- clamps, 4- screws, 5-support, 6-rod, 7-moving yoke, 8-metallic thread, 9- beam, 10- counterbalance, 11-electromagnets, 12- mirror , 13- source of light ray, 14- mirror, 15 – scale, 16 - heat chamber.

The measuring methods is detailed in [10]. The experimental methods provides to obtain the shear modulus *G* during forming of gel.

The view of clamps with cuvette is represented in Fig.3



a b

Fig.3. The cuvette between clamps: a) view of cuvett bettwem clamps, b) the diagram of sample loading

The cuvette with the substance being investigated undergoes the action of the twisting moment M

(Fig.2b). Heating is produced with the aid of the thermostat into the walls of heat chamber.

In experiment the object of research is the water solution of natively of al'bumenu (native albumen) with the concentration of al'bumenu 12 %. Native al'bumen is the mixture of globular proteins. The basic component of such mixture is oval'bumin . His part in al'bumeni is considerable and is 69% [11].

Experimental results and discussion

The experimental dependence for ovalbumin during denaturation $G(t)$ is represented in Fig.4.

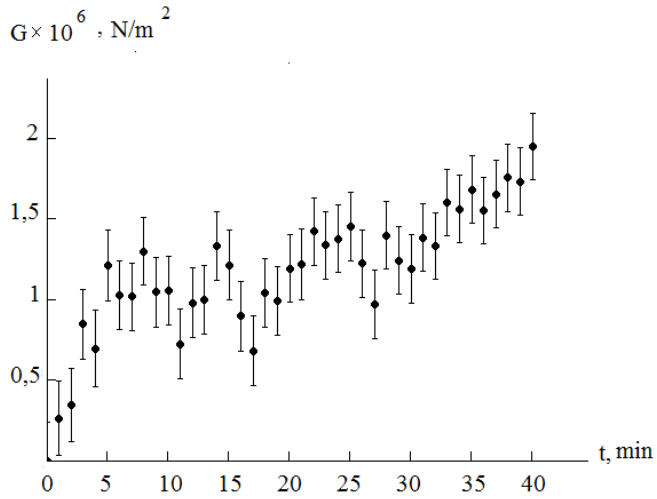


Fig.4. The experimental time dependence $G(t)$ for native albumen during ovalbumin denaturation at the temperature of hyperpyrexia 42ºC.

As can be seen from this figure the process of ovalbumin denaturation constists of two stages: during the first stage is observed an alternation in the module in the course of time, on the second - monotonic growth of module in the course of time. The duration of the first stage is 27 minutes. An alternation in shear modulus is during the first stage irregular phenomenon. With which is connected the periodicity indicated?

As is known, in the initial stage of denaturation [12] remains the possibility of reverse process is renaturation. This point of view the observed periodicity can explain by the fact that at the some moments of time first the denaturation predominates on the renaturation , then renaturation predominates above the denaturation. This possibility appears, apparently in connection with the fact that the equation, which describes denaturation and renaturation contain the nonlinear terms, which connect these equations. Therefore the observed periodicity appears: it is conventionally designated as temporary dissipative structure. Its appearance is the manifestation of self-organizing protein [12].

This treatment of the observed periodicity makes it possible to assert that with the presence of periodicity is connected from the reversible denaturation of protein. Since in the second stage of denaturation the periodicity is absent to logical assert that the reversible denaturation is impossible in this stage. So that the duration of the first stage is the period of time, in which are still possible reversed denaturation. So the period of the reversible denaturation of ovalbumin is equal to 27 minutes.

For comparison the light-scattering in the native albumen during ovalbumin denaturation was investigational using traditional nephelometric method [13]. The results are represented in Fig.5. The value ξ_g is the rationed intensity which equals the relation of the intensity of light –scattering in arbitrary moment of time $I(t)$ to intensity of light-scattering in initial moment of time I_0.

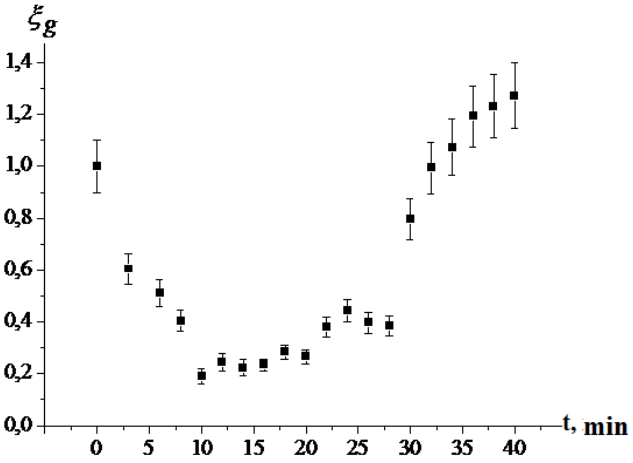


Fig. 5. Light-scattering in solution of native albumen during ovalbumin denaturation at the temperature of hyperpyrexia (42ºC).

Comparison of dependences on Fig.4 and Fig.5 specifies to the coinciding of the time interval of renaturation stage.

Conclusions

The new method of studying the denaturing of globular proteins is proposed. The period of the reversible denaturation of globular proteins makes possible to determine this method. On the example of ovalbumin as the models of globular proteins is established that the period of the reversible denaturation at temperature of hyperpyrexia (42ºC) composes 27 minutes.

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