МОЛЕКУЛЯРНА БІОФІЗИКА

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FRACTAL TEXTURES OF BOVINE SERUM ALBUMIN FILMS

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The texture features of the films obtained from solutions of bovine serum albumin (BSA) in the presence of sodium and copper ions are considered. The apparatus for producing films and the absorption spectra of BSA in the UV wavelength range are presented. The films have been received from BSA solutions with concentration of 0.41 mg/ml in the presence of salt NaCl at a concentration of 20 mM and in the presence of salt $CuCl_2$ with concentrations of 0.1 mM and 0.4 mM. Texture films of BSA, human serum albumin and calf thymus DNA are compared. The obtained scale invariant fractal textures are similar to textures which are formed on the films surface from the DNA solutions. Presence in solution of heavy metal ions forming compounds with albumin molecules prevents the formation of scale invariant fractal textures on films from these solutions.
KEY WORDS: solution, film, albumin, fractal, texture.

ТЕКСТУРИ ПЛІВОК СИРОВАТКОВОГО АЛЬБУМІНУ БИКА

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У роботі розглядаються особливості текстур плівок, отриманих з розчинів бичачого сироваткового альбуміну (БСА) у присутності іонів натрію і міді. Представлені пристрій для отримання плівок і спектри поглинання розчинів бичачого сироваткового альбуміну БСА в ультрафіолетовому діапазоні довжин хвиль. Плівки були отримані з розчинів бичачого сироваткового альбуміну (БСА) з концентрацією 0.41 мг/мл у присутності солі NaCl з концентрацією 20 мМ, а також у присутності солі $CuCl_2$ з концентраціями 0,1 мМ і 0,4 мМ. Порівнюються текстури плівок БСА, сироваткового альбуміну людини і ДНК тимуса теляти. Отримані масштабно інваріантні фрактальні текстури аналогічні текстурам, які формуються на поверхні плівок з розчинів ДНК. Присутність в розчинах іонів важких металів, що утворюють сполуки з молекулами БСА перешкоджає формуванню масштабно інваріантних фрактальних текстур на плівках.
КЛЮЧОВІ СЛОВА: розчин, плівка, альбумін, фрактал, текстура.

ТЕКСТУРЫ ПЛЕНОК СЫВОРОТОЧНОГО АЛЬБУМИНА БЫКА

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В работе рассматриваются особенности текстур пленок, полученных из растворов бычьего сывороточного альбумина (БСА) в присутствии ионов натрия и меди. Представлены устройство для получения пленок и спектры поглощения растворов бычьего сывороточного альбумина БСА в ультрафиолетовом диапазоне длин волн. Пленки были получены из растворов бычьего сывороточного альбумина (БСА) с концентрацией 0.41 мг/мл в присутствии соли NaCl с концентрацией 20 мМ, а также в присутствии соли $CuCl_2$. с концентрациями 0,1 мМ и 0,4 мМ. Сравниваются текстуры пленок БСА, сывороточного альбумина человека и ДНК тимуса теленка. Полученные масштабно инвариантные фрактальные текстуры аналогичны текстурам, которые формируются на поверхности пленок из растворов ДНК. Присутствие в растворах ионов тяжелых металлов, образующих соединения с молекулами БСА препятствует формированию масштабно инвариантных фрактальных текстур на пленках.
КЛЮЧЕВЫЕ СЛОВА: раствор, пленка, альбумин, фрактал, текстура.

Theoretical and experimental studies of the solid phase of various biological liquids (BL) including films from solutions of biopolymers have been developed in recent years. So, the method of diagnostic testing for detecting metastatic carcinoma by means of studying the topology of the drying plasma protein is proposed in the work [1], methods for studying pathological changes on the structures observed as a result of dehydration self-organization of BL are considered in [2]. A method of diagnosing of cerebrospinal fluid based on the study of texture of dried samples is described in the [3].

However, in the work [4] it is noted that most of the methods is considered at the stage of phenomenological description complicating the statistical analysis of the data. On the other hand, in the paper [5] it is shown that the processes of dehydration self-organization of the BL can be described in terms of the standard approaches, inherent in physical and chemical methods of the research. For example, the stages of the process of structuring at BL drying are considered in the work [6], the relevant theoretical models of behavior of spatial redistribution of the components of drying droplet of BL are created in the work [7]. Formation of the textures on the film obtained from DNA containing solutions with taking into account interaction of charges in DNA ion-phosphate lattice and the metal ions is grounded in [8, 9]. Studies of the effect of biologically active substances (metal ions) on formation of the textures of the films from DNA's solutions were carried out before [10]. It has been shown that the character of the formation of dendrites on the surface of the film is determined by the structural state of the biopolymer [11].

The texture features of the films obtained from solutions of bovine serum albumin (BSA) in the presence of sodium and copper ions are considered in the paper.

MATERIALS AND METHODS

Schematic representation of the apparatus for producing films textures from biopolymer solutions is presented in Fig. 1. The solutions of biopolymers with volume 0.5 ml were poured into cuvette measuring 20 * 21 mm^2, which is located on the stand 1. The cuvette is in container 5 with silica gel 2 and humidity sensor 3 "HCH-1000" ("Honeywell"), display control of the humidity sensor was carried out by using LCR - meter "E7-11." The container 5 is positioned in the water thermostat TX-50. The films have been received from BSA (98%) and human serum albumin (HAS, 85%) solutions from "Sigma-Aldrich" with concentration of 0.41 mg/ml in the presence of salt NaCl at a concentration of 20 mM and in the presence of salt $CuCl_2$ with concentrations of 0.1 mM and 0.4 mM.

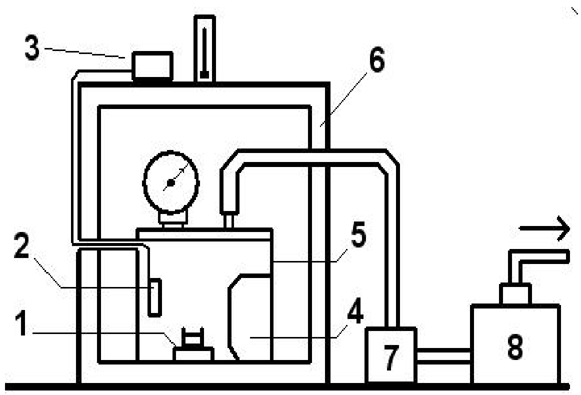

Fig. 1. The apparatus for producing films.
1 - Stand with cuvette for solution, 2 – humidity sensor, 3 - LCR meter E7-11, 4 - container with silica gel, 5 - airtight container, 6 - water thermostat, 7 - vacuum valve, 8 - vacuum pump.

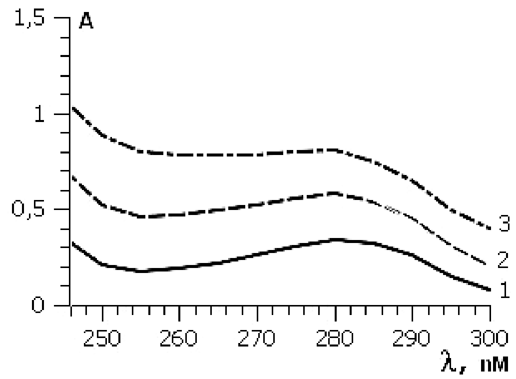

Fig. 2. The absorption spectra of BSA solutions in the UV wavelength range, comprising:
A_{BSA} - solid line, BSA + 20 mM NaCl;
$A_{0.1Cu}$ - dotted line, BSA + 20 mM NaCl + 0.1 mM CuCl2;
$A_{0.4Cu}$ - dash-dotted line

The films have been received also from solutions of calf thymus Na-DNA from "Serva" with concentration of 0.25 mg/ml in the presence of salt NaCl with concentration of 10 mM using the apparatus described in the [12]. Measurements of the solution absorption in the UV wavelengths were performed with UV-2100 Spectrometer of "UNICO," the measurement absorption error was of δA = ± 1%. The calculation of protein concentration according to BSA absorption in the UV range at wavelengths of 260 and 280 nm (curve 1 in Fig. 2) by the method [13] shows the concentration value of 0.37 mg/ml. The films obtained at 0% humidity were photographed in polarized light. The accuracy of temperature stabilization is 0.5ºC, the accuracy of humidity determination is 2-3%.

RESULTS AND DISCUSSION

The photos of the film obtained from BSA solution are represented in Fig. 3 and 4. The obtained scale invariant fractal textures (SIFT) are similar to textures which are formed on the films surface of the DNA solutions with salts of sodium chloride. Fragments of the texture are shown in Fig. 5 and 6. Similar textures can be obtained from DNA solutions with values of relative humidity from 11 to 40% [12] as well as from solutions of human serum albumin, as it is noted in the report [14]. Formation of SIFT can be represented as the series connection of elementary cell structures with characteristic angular α and linear β parameters (Fig. 7), which form a different scale patterns on the film surface. The dimensions of these structures may be in the range of 90º <α <180° and 2 <β <35 μm.

It can be assumed that some of the ordered associative structures from molecules of BSA, water and metal ions are formed in the colloidal solution in the evaporation process. The metal salts do not form a fractal pattern on the surface of the cell [9]. Thus, the formed patterns remain after evaporation of the water. A similar assumption on the sequence of structure formation on the film is made in [6]. In [14] it is assumed that the polyelectrolyte supramolecular structure can be induced by hydrogen bonding.

Copper ions form stable complexes with BSA molecules due to the fact that the BSA has a specific binding site for these ions [16-18], that films have not SIFT (Fig. 8).

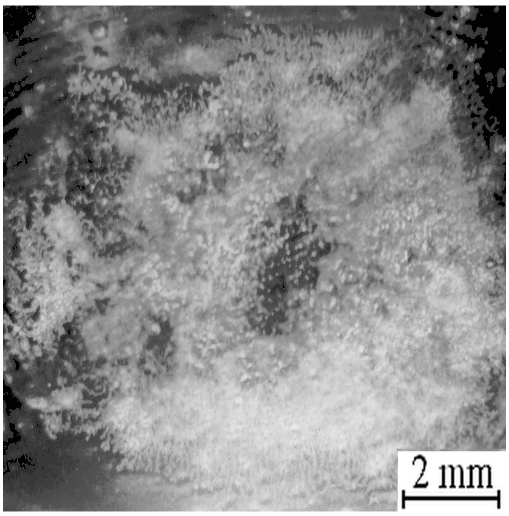

Fig. 3. Photo of the film obtained from solution containing BSA + 20 mM NaCl. T=40C, RH=0%.

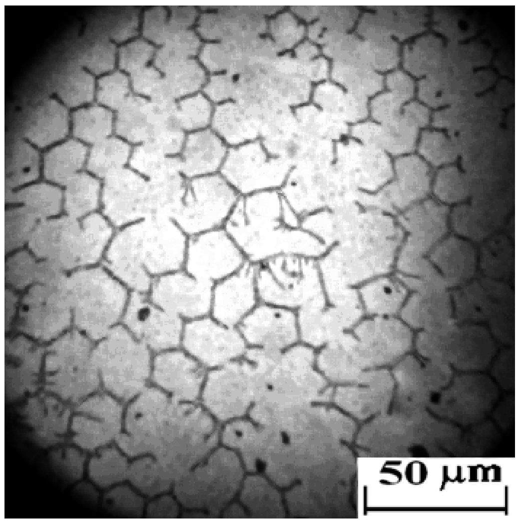

Fig. 4. Photo of the film obtained from solution containing BSA + 20 mM NaCl. T=40C, RH=0%.

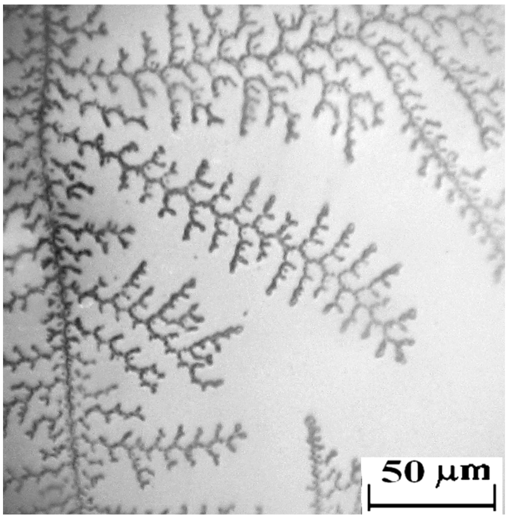

Fig. 5. Photo of the film obtained from solution containing Na-DNA + 10 mM NaCl. T=30C, RH=46%.

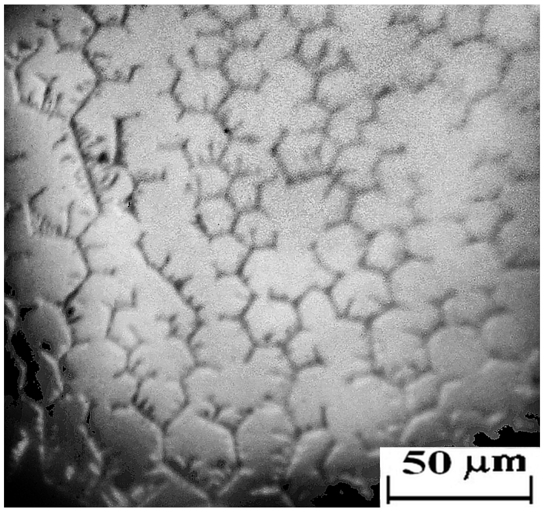

Fig. 6. Photo of the film obtained from solution containing HSA + 20 mM NaCl. T=40C, RH=0%.

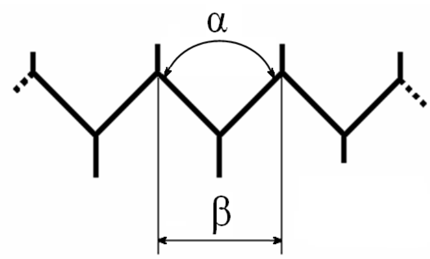

Fig. 7. Characteristic geometric parameters of SIFT.

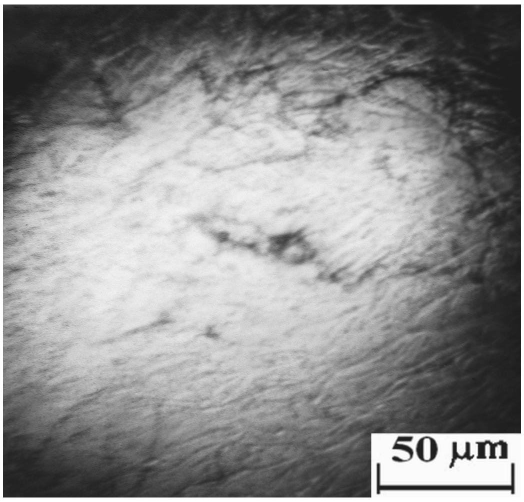

Fig. 8. Photo of the film obtained from solution containing BSA + 20 mM NaCl +0.1 mM $CuCl_2$. T=40C, RH=0%.

Changing the values of the spectra absorption ($\Delta A = A_{0.1Cu} - A_{BSA}$ and $\Delta A = A_{0.1Cu} - A_{BSA}$, Fig. 2) in the UV range can be regarded as a measure of effect of copper ions on BSA molecule [19]. Similarly, the method based on the change in film texture area can be used to determine the effect [20].

The absence of the SIFT on the BSA films can be explained by the formation of complexes of copper ions with BSA and destruction of the respective hydrogen bonds, which are necessary for the formation of that textures. It can be concluded that the presence in solution of heavy metal ions forming compounds with BSA molecules prevents the formation of scale-invariant fractal textures on films from these solutions.

REFERENCES

1. Рапис Е. Г. Изменение физической фазы неравновесной пленки комплекса белков плазмы крови у больных с карциномой / Е. Г. Рапис // Журнал технической физики. – 2002. – № 4. – С. 139–142. /Rapis E. G. Izmenenie fizicheskoj fazy neravnovesnoj plenki kompleksa belkov plazmy krovi u bol'nyh s karcinomoj / E. G. Rapis // Zhurnal tehnicheskoj fiziki. – 2002. – № 4. – S. 139–142./

2. Швецова Н.А. Диагностика заболеваний по картине самоорганизации в высыхающих каплях биологических жидкостей / Н.А. Швецова // Биомедицинская радиоэлектроника. – 2012. – №3. – С. 30-36. /Shvecova N.A. Diagnostika zabolevanij po kartine samoorganizacii v vysyhajushhih kapljah biologicheskih zhidkostej / N.A. Shvecova // Biomedicinskaja radiojelektronika. – 2012. – №3. – S. 30-36./
3. Шатохина С. Н. Маркер дискогенных заболеваний нервной системы в цереброспинальной жидкости / Н. Шатохина, В. С. Кузнецова, В. Н. Шабалин // Журнал неврологии и психиатрии им.С.С.Корсакова. – 2011. – № 12. С. 4–8. /Shatohina S. N. Marker diskogennyh zabolevanij nervnoj sistemy v cerebrospinal'noj zhidkosti / N. Shatohina, V. S. Kuznecova, V. N. Shabalin // Zhurnal nevrologii i psihiatrii im.S.S.Korsakova. – 2011. – № 12. S. 4–8./
4. Maksimov S. A. Morphology of the solid phase of biological liquids as method of diagnostics in medicine / S. A. Maksimov // Bulletin of Siberian medicine. – 2007. – № 4. – P.80–83.
5. Тарасевич Ю. Ю. Механизмы и модели дегидратационной самоорганизации биологи¬ческих жидкостей / Ю. Ю. Тарасевич // Успехи физических наук. – 2004. – №7. – С. 779–790. /Tarasevich Ju. Ju. Mehanizmy i modeli degidratacionnoj samoorganizacii biologi¬cheskih zhidkostej / Ju. Ju. Tarasevich // Uspehi fizicheskih nauk. – 2004. – №7. – S. 779–790./
6. Белок и соль: пространственно-временные события в высыхающей капле / Т. А., Яхно, В. Г. Яхно, А. Г. Санин [и др.] // ЖТФ, 2004. – №8. – С. 100–108. /Belok i sol': prostranstvenno-vremennye sobytija v vysyhajushhej kaple / T. A., Jahno, V. G. Jahno, A. G. Sanin [i dr.] // ZhTF, 2004. – №8. – S. 100–108./
7. Tarasevich Y.Y. Modeling of spatial-temporal distribution of the components in the drying sessile droplet of biological fluid / Y.Y. Tarasevich, I.V. Vodolazskaya, O.P. Bondarenko // Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2013. Vol. 432.— P. 99–103.
8. Mayeres, C. H.; Lee, S. A.; Pinnick, D. A.; Carter, B. J.; Kim, J. A study of Na-DNA films containing NaCl via scanning electron and tunneling microscopies / C. H. Mayeres [et al] // Biopolymers, 1995. – Vol. 36. – P. 669–673.
9. Perepelytsya S.M. Texture formation in DNA films with alkali metal chlorides / S.M. Perepelytsya G.M. Glibitskiy, S.N. Volkov // Biopolymers, 2013. Vol. 99. – P. 508–16.
10. Глибицкий Г. М. Текстуры пленок Na-ДНК, полученные из растворов, содержащих ТРИС и ЭДТА / Г. М. Глибицкий, М. А. Семенов, Д. М. Глибицкий // Біофізичний вісник – 2011. – №2. – С. 26–30. /Glibickij G. M. Tekstury plenok Na-DNK, poluchennye iz rastvorov, soderzhashhih TRIS i JeDTA / G. M. Glibickij, M. A. Semenov, D. M. Glibickij // Biofizichnij visnik – 2011. – №2. – S. 26–30./
11. Glibitskiy G.M. Interaction of DNA with Silver Nanoparticles / G.M. Glibitskiy, V.V. Jelali, M.O. Semenov [et al.] // Ukr. J. Phys., 2012. – Vol. 7. –P.695–699.
12. Glibitskiy G.M. Structures of Na-DNA films received from solutions at various values of humidity and temperatures / G.M. Glibitskiy, A.A. Krasnitskaja, V.I. Gudzenko// Biophysical Bulletin, 2006. –Vol. 1. – P. 89–94.
13. Степанченко Н. С. Количественное определение содержания белка / Н. С. Степанченко, Г. В. Новикова, И. Е. Мошков // Физиология растений. – 2011. – № 4. – С.624–630. /Stepanchenko N. S. Kolichestvennoe opredelenie soderzhanija belka / N. S. Stepanchenko, G. V. Novikova, I. E. Moshkov // Fiziologija rastenij. – 2011. – № 4. – C.624–630./
14. Glibitskiy G.M. // Nanoboiphysics: “Fundamental and Applied Aspects. Third International Conference.” – Kharkiv:- Publishing “B.Verkin Institute for Low Temperature Physics and Engineering,” 2013. – P.109.
15. Kotz J. Self-assembled polyelectrolyte systems / J. Kotz, S. Kosmella, T. Beitz. // Prog. Polym. Sei. – 2001. Vol. 26. – P. 1199–1232.
16. Peters T. Copper-binding Properties of Bovine Serum Albumin and Its Amino-terminal Peptide Fragment / T. Peters, F. A. Blumenstock // Journ. Biol. Chem. – 1967. –Vol. 242. – P. 1574–1578.
17. Impact of copper to the chelation effect of bovine serum albumin and spermatozoa motility parameter in vitro / Z. Kňažická, J. Lukáčová, E. Tvrdá [et al] //Contemporary Agriculture, 2012. Vol. 61. – P. 15-28.
18. Short peptides are not reliable models of thermodynamic and kinetic properties of the N-terminal metal binding site in serum albumin / Sokolowska M., Krezel1 A., Dyba1 [et al] / Eur. J. Biochem., 2002. – Vol. 269. – P. 1323–1331.
19. Farruggia B. G. Pico. Bile Salts Binding-Induced Alterations in Ultraviolet Absorption Spectrum of Bovine Serum Albumin / B. Farruggia, G. Pico // Gen. Physiol. Biophys. – 1992. – Vol. 11. – P. 4–218.
20. Glibitskiy G.M., Krasnitskaya A.A. Authors’ certificate N 86246 UA, Bull. N.7. (10.04.2009).