МОЛЕКУЛЯРНА БІОФІЗИКА

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TEXTURES OF BSA FILMS WITH IRON AND COPPER IONS

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Analysis of the patterns on the dried films of biological fluids is a promising avenue of medical diagnostics and biosensors development. The aim of this work was to study the effect of iron and copper ions on the quantitative characteristics of textures and zigzag patterns (Z-structures) observed on the films of saline solutions of bovine serum albumin (BSA). In the experiment, 0.5 mg/ml BSA, 20 mM/l NaCl, 0.05 mM/l $CuCl_2$ and 0.2÷0.05 mM/l $FeCl_3$ were used. For textures, the area and fractal dimension were estimated, and for zigzag patterns, area and the specific length of the Z-structures were estimated. The effect of iron and copper ions on the geometrical parameters of Z-structures was analyzed. Spatial distributions of the fractal dimension of texture and the specific length of Z-structures were analyzed. It was shown that using the product of all four parameters increases the sensitivity of the method compared to using only one of the parameters. The method can be used in pharmacology and medical biochemistry for screening drugs and chemical compounds by their degree of influence on biopolymers.
KEY WORDS: solution, film, texture, biopolymer, fractals, biologically active substances.

ТЕКСТУРИ ПЛІВОК БСА З ІОНАМИ ЗАЛІЗА ТА МІДІ

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Аналіз патернів на висушених плівках біологічних рідин є перспективним напрямком медичної діагностики і розробки біосенсорів. Метою цієї роботи було вивчення впливу іонів заліза і міді на кількісні характеристики текстур і зигзагоподібних патернів (Z-структур), які спостерігаються на плівках сольових розчинів бичачого сироваткового альбуміну (БСА). У роботі були використані 0,5 мг/мл БСА, 20 мМ/л NaCl, 0,05 мМ/л $CuCl_2$ і 0,05÷0,2 мМ/л $FeCl_3$. Для текстур оцінювалися площа і фрактальна розмірність, а для зигзагоподібних патернів – площа і питома довжина Z-структур. Були проаналізовані відмінності геометричних параметрів Z-структур при впливі іонів заліза і міді. Були проаналізовані просторові розподіли фрактальної розмірності текстур і питомої довжини Z-структур. Було показано, що використання добутку всіх чотирьох параметрів підвищує чутливість методу в порівнянні з використанням тільки одного з параметрів. Цей метод може застосовуватись у фармакології та медичній біохімії для визначення ступеня впливу препаратів та хімічних речовин на біополімери.
КЛЮЧОВІ СЛОВА: розчин, плівка, текстура, біополімер, фрактали, біологічно активні речовини.

ТЕКСТУРЫ ПЛЕНОК БСА С ИОНАМИ ЖЕЛЕЗА И МЕДИ

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Анализ паттернов на высушенных пленках биологических жидкостей является перспективным направлением медицинской диагностики и разработки биосенсоров. Целью данной работы было изучение влияния ионов железа и меди на количественные характеристики текстур и зигзагоподобных паттернов (Z-структур), наблюдаемых на пленках солевых растворов бычьего сывороточного альбумина (БСА). В работе были использованы 0,5 мг/мл БСА, 20 мМ/л NaCl, 0,05 мМ/л $CuCl_2$ и 0,05÷0,2 мМ/л $FeCl_3$. Для текстур оценивались площадь и фрактальная размерность, а для зигзагоподобных паттернов – площадь и удельная длина Z-структур. Были проанализированы различия геометрических параметров Z-структур при воздействии ионов железа и меди. Были проанализированы пространственные распределения фрактальной размерности текстур и удельной длины Z-структур. Было показано, что использование произведения всех четырех параметров повышает чувствительность метода по сравнению с

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использованием только одного из параметров. Этот метод может применяться в фармакологии и медицинской биохимии для определения степени влияния препаратов и химических веществ на биополимеры.
КЛЮЧЕВЫЕ СЛОВА: раствор, пленка, текстура, биополимер, фракталы, биологически активные вещества.

Methods of analysis of film textures obtained from solutions of biological fluids are used to create new medical technologies [1] and for diagnosis of various diseases. For example, the authors of [1] and [2] provide an overview of medical research methods for qualitatively assessing the morphology of the solid phase of biological fluids. On the other hand, methods for quantitative analysis of texture are also being developed. For example, in [3] the main types of structural elements observed in the course of dehydration are analyzed, which are then used for quantitative evaluation based on pattern recognition. In [4], the authors investigate the textures of films obtained from chitosan solution in the presence of copper and silver ions, using fractal dimension calculations to evaluate the observed patterns. This lets one compare the observed structures with some well-known aggregation models.

Previously, we have analyzed the impact of biologically active substances (BAS) on biopolymers in relation to the changes to the area and the fractal dimension of the film texture [5-7]. Another criterion for estimating the impact of BAS was proposed in [8], based on the relative area occupied by zigzag patterns (Z-structures). However, this method has insufficient sensitivity, since the area calculations cannot easily discern densely-packed Z-structures from each other or from other types of patterns which lie in close proximity. To mitigate the issue, a direct calculation of the specific density of Z-structures was employed, which was used in [9] to show that Z-structures do not form in case of thermal denaturation of bovine serum albumin (BSA).

The aim of this work is to apply the Z-structure based methods of quantitative evaluation to investigate the film textures obtained from the solutions of BSA and iron or copper ions.

MATERIALS AND METHODS

The films were prepared from solutions of bovine serum albumin ("Sigma-Aldrich") at a concentration of 0.5 mg/ml in the presence of NaCl at a concentration of 20 mM. Chemically pure reagents ($CuCl_2$, $FeCl_3 \cdot 6H_2O$) were used. The setup for the production of films and the method of texture formation on the film at 0% relative humidity are described in [10].

For a biopolymer film obtained from the solution of the biopolymer and the studied BAS, an influence coefficient K_T representing the area of textures on the film is determined [5]:

$$K_T = S_T / S \,, \tag{1}$$

where S_T is the area of textures on the film, and S is the area of the entire film.

One of the parameters used for the analysis of textures is the fractal dimension D, which characterizes the degree of branching or irregularity of the texture. Generally, D is expressed as:

$$D = \lim_{\varepsilon \to \infty} \frac{\log N(\varepsilon)}{\log(\varepsilon)}, \tag{2}$$

where N is the number of elements (squares, circles, etc.) sufficient to cover the Z-structures, and ε is the linear element size.

If it is impossible to clearly separate the background from the structures, a variant of fractal dimension is used which takes the "intensity" of the image into account:

$$D = \lim_{\varepsilon \to \infty} \frac{\log X(\varepsilon)}{\log(\varepsilon)}, \tag{3}$$

where X is a quantitative characteristic of the element content.

For the calculation of fractal dimension, the "differential volume variation" box counting method was used. For each value of ε (from a predetermined set of scales), the image is covered with an ε-sized square grid. For each square, an intensity is calculated:

$$I_\varepsilon = \sum_{i,j} (1 + \partial I_{i,j,\varepsilon}), \tag{4}$$

where $\partial I_{i,j,\varepsilon}$ is the difference between the maximum and minimum "intensity" of the image within a given square. I_ε corresponds to the "volume" $V_\varepsilon = I_\varepsilon \cdot \varepsilon^2$. For the plot of log$(V)$ against $\log(\varepsilon)$, the slope of the regression line is calculated as

$$S = \lim_{\varepsilon \to 0} \frac{\ln V_\varepsilon}{\ln 1/\varepsilon}, \tag{5}$$

and the fractal dimension is calculated as

$$D = 3 - (S/2). \tag{6}$$

The coefficient K_{Z_S} accounts for the area occupied by the Z-structures:

$$K_{Z_S} = S_Z / S, \tag{7}$$

where S_Z is the area of Z-structures on the film.

The coefficient K_{Z_L} accounts for the length of the individual Z-structures:

$$K_{Z\_L} = \frac{\sum \left(L_{(z)i} / S_{(z)i}\right)}{n} \tag{8}$$

where $L_{(z)i}$ is the total length of Z-structures in the i-th sample (micrograph) of the film; n is the number of the taken samples, and $S_{(z)i}$ is the area of the i-th sample. The micrographs are taken at the locations uniformly distributed over the film area in a grid fashion.

The effect P of the substance is defined as the product of all the calculated coefficients:

$$P = K_T \cdot D \cdot K_{Z_S} \cdot K_{Z_L}. \tag{9}$$

RESULTS AND DISCUSSION

Examples of film micrographs are presented in Fig. 1a (BSA and 20 mM NaCl), Fig. 1b (BSA, 20 mM NaCl and 0.05 mM $FeCl_3$), Fig. 1c (BSA, 20 mM NaCl and 0.05 mM $CuCl_2$) and Fig. 1d (BSA, 20 mM NaCl and 0.1 mM $FeCl_3$). The values of specific length L_S ($L_{(z)i} / S_{(z)i}$), average edge length L_E and average angle A of Z-structures for the example micrographs are: L_S =18.5 mm^{-1}, L_E =0.08±0.06 mm, A =116±23° for Fig. 1a (control film); L_S =17.2 mm^{-1}, L_E =0.04±0.01 mm, A =117±15° for Fig. 1b (0.05 mM $FeCl_3$); L_S =10.3 mm^{-1}, L_E =0.07±0.03 mm, A =118±12° for Fig. 1c (0.05 mM $CuCl_2$); L_S =20.4 mm^{-1}, L_E =0.04±0.02 mm, A =118±20° for Fig. 1d (0.1 mM $FeCl_3$). Z-structures with the parameters similar to those of Fig. 1a (control film) are also present in the central regions of the films with copper and iron ions.

Fig. 1a is characterized by the longest zigzag edges and a high amount of side-branches; Fig. 1b-1d show a more hexagonal layout of zigzags with little to no side-branching; finally, for Fig. 1c, Z-structures appear to be larger and more sparse than for Fig. 1b and 1d. As the concentration of $FeCl_3$ increases, the film becomes increasingly dominated by DLA patterns, dense branching patterns and disordered thread-like patterns (not shown).

The area occupied by Z-structures was calculated as the area of a minimum bounding polygon which contains the visible elements of Z-structures. An example is shown in Fig. 1a, where label 1 marks the bounding polygon, label 2 marks the Z-structures and label 3 marks non-Z-structures. As such, these area calculations do not take into account the density of Z-structures and the presence of different types of patterns inside the area.

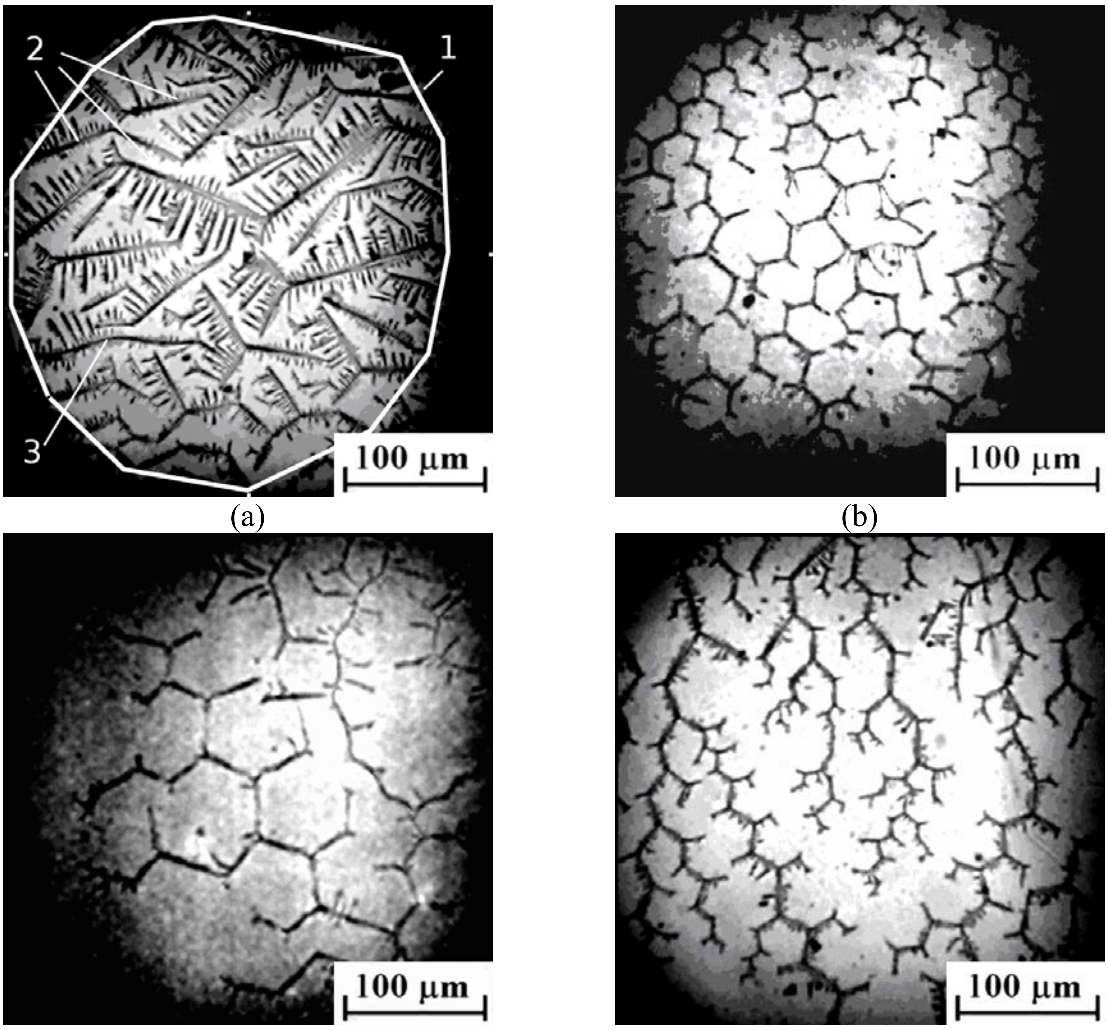

Fig. 1. Example micrographs of films corresponding to the solutions of: (a) BSA and 20 mM NaCl; (b) BSA, 20 mM NaCl and 0.05 мM $FeCl_3$; (c) BSA, 20 mM NaCl and 0.05 $CuCl_2$; (d) BSA, 20 mM NaCl and 0.1 мM $FeCl_3$.

The distributions of specific length (L_S) of Z-structures and fractal dimension (D) of the textures are shown in Fig. 2a and Fig. 3a (control film), Fig. 2b and Fig. 3b (0.05 mM $FeCl_3$), Fig. 2c and Fig. 3c (0.05 mM $CuCl_2$), respectively. It can be observed that zigzag patterns tend to only appear at approximately 3 mm distance from both the center and the borders of the cell. This might be due to local anisotropy and the distribution of salt and BSA (determined by the interplay of diffusion, radial flow, DLVO (Derjaguin, Landau, Verwey, and Overbeek) force and Marangoni recirculation loop, as well as the hydrodynamic effects during the late-stage drying) [11-18] creating the necessary conditions for Z-structure formation only at a 3–7 mm distance from the cell borders. The fractal dimension for

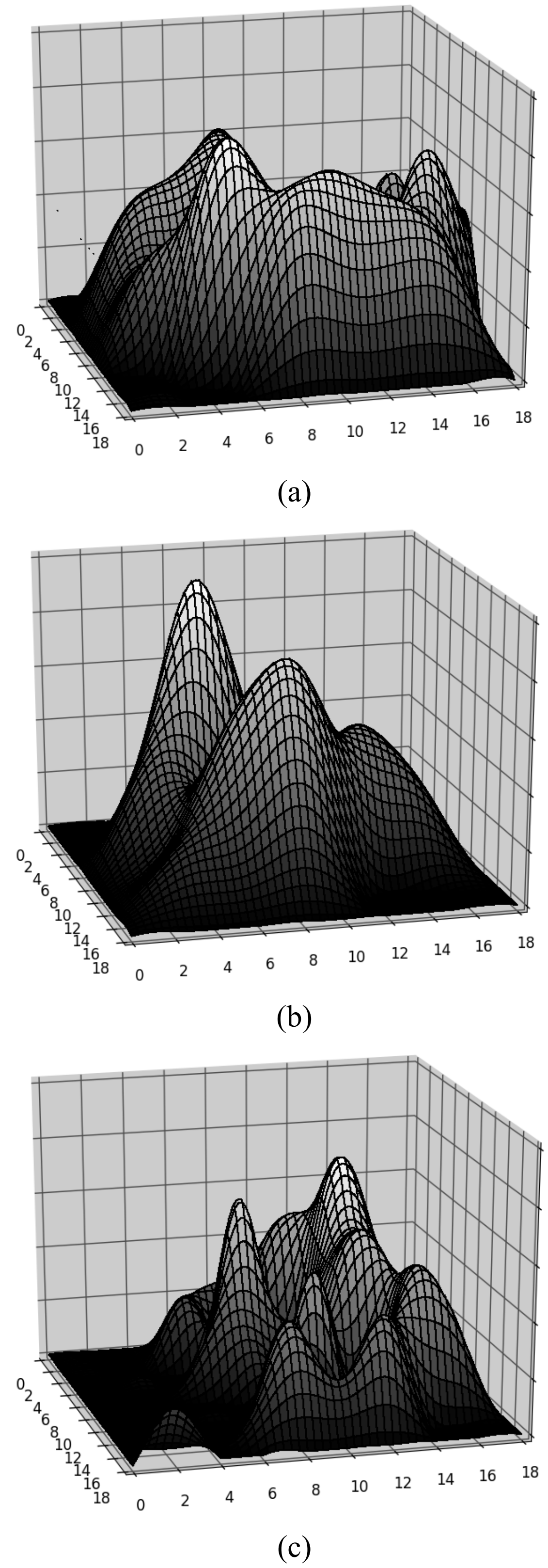

Fig. 2. Averaged distributions of specific lengths of Z-structures on films corresponding to the solutions of: (a) BSA and 20 mM NaCl; (b) BSA, 20 mM NaCl and 0.05 мM $FeCl_3$; (c) BSA, 20 mM NaCl and 0.05 $CuCl_2$.

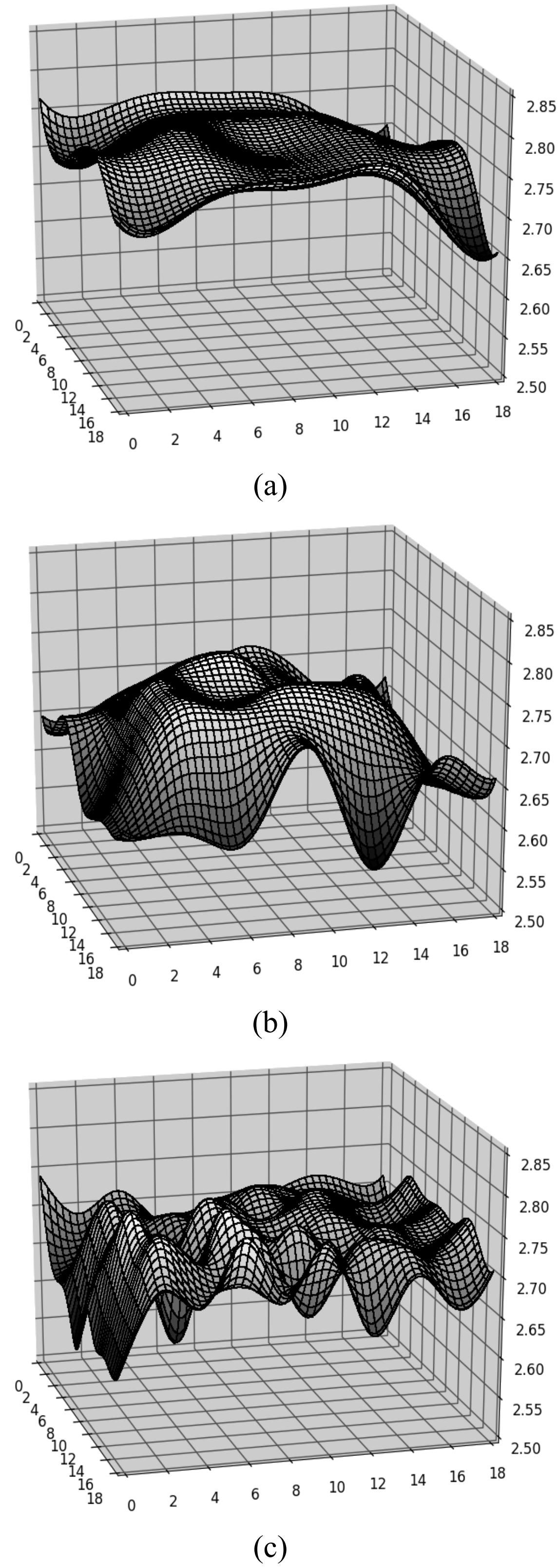

Fig. 3. Averaged distributions of texture fractal dimension on films corresponding to the solutions of: (a) BSA and 20 mM NaCl; (b) BSA, 20 mM NaCl and 0.05 мM $FeCl_3$; (c) BSA, 20 mM NaCl and 0.05 $CuCl_2$.

0.05 mM $CuCl_2$ films shows little difference between the center and the borders, whereas for 0.05 mM $FeCl_3$ film, less complex aggregation patterns tend to appear at the cell borders.

The mean values of each evaluated parameter are provided in Table 1 for each of the investigated solutions. For comparison, the P values calculated using our current method (P_{new}) and the previous method (P_{prev}), which considered only K_T, D and K_{Z_S}, are provided.

Table 1

The individual coefficients and the resulting effect estimation of each solution

Solution	K_T Δ=5%	D Δ=5%	K_{Z_S} Δ=10%	K_{Z_L}, mm^{-1} Δ=10%	P_{prev}, mm^{-1} Δ=16%	P_{new}, mm^{-1} Δ=16%
BSA+20 mM NaCl	0,98	2,71	0,53	7,2	1,41	10,1
BSA+20 mM NaCl +0,05 mM $FeCl_3$	0,90	2,68	0,34	6,3	0,82	5,2
BSA+20 mM NaCl +0,05 mM $CuCl_2$	0,95	2,70	0,34	7,1	0,87	6,2
BSA+20 mM NaCl +0,10 mM $FeCl_3$	0,84	2,67	0,46	7,2	1,03	7,4
BSA+20 mM NaCl +0,20 mM $FeCl_3$	0,95	2,74	0,15	2,9	1,34	1,1

The table shows that the coefficients K_T and D change very little for the Fe^{3+} concentration range of 0 to 0.20 mM (the variation is within the bounds of error), while the coefficients K_{Z_S} and K_{Z_L} decrease by more than a factor of 3 and 2, respectively, and P_{new} decreases by about a factor of 9. The use of all the coefficients in the formula (9) results in an increased sensitivity compared to the use of only one of the coefficients, as well as compared to the use of the previous formula.

As was shown in [10], the formation of fractal patterns on the surface of biopolymer films can be explained by the formation of crystal hydrate complexes of water, protein and salt. It can be assumed that the addition of copper or iron ions disturbs the conditions of crystal hydrate formation, which reduces the amount of Z-structures on the film surface. A detailed explanation of the mechanism of Z-structure formation is the subject of a separate study.

CONCLUSIONS

A method for the analysis of BAS influence on biopolymers is proposed, based on the property of biopolymer solutions with different composition to form different textures during the evaporation. The experimental study was conducted using bovine serum albumin as the biopolymer, and iron and copper salts as the BAS. Four parameters of the textures were analyzed: the texture area, the fractal dimension, the area of Z-structures and the specific length of Z-structures. Using the product of all four parameters increased the sensitivity of the method compared to using only one of the parameters. The method can be used in pharmacology and medical biochemistry for screening drugs and chemical compounds by their degree of influence on biopolymers.

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