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Ellipsometrical and SEM Study of Ferritin Adsorption on a Gold Surface

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The adsorption of horse spleen ferritin on gold surface is studied with null ellipsometry and scanning electron microscopy. The high sensitivity and *in situ* advantage of ellipsometry make it very attractive for studies of ultrathin biomolecular layers. The influence of pH and protein concentration in solution on the adsorption kinetics was interpreted using *ex situ* scanning electron microscopy shots and computer modeling of the system "prism–gold film–adsorbed layer–solution". It seems that the first fast stage comprises nucleation process occurring at active sites and the following slow multistage process of a protein film growth. The data about adsorption kinetics of protein and the structure of the adsorbate layers obtained in this study enable understanding of such processes in real biological system, e.g. the protein adsorption on blood vessels and tissues in pathological processes.

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1. Introduction

Investigation of such surface phenomena as critical nuclei formation and film growth during adsorption gives us a key to understand a lot of processes important for biomaterials, biosensors, and biotechnology [1].

In the last years many experimental and theoretical papers were published [2–6], concerning the kinetics of adsorption in simple model systems. It is commonly assumed that the protein molecules repeatedly impinge upon the surface, before they adsorb on it [7]. According to this model the molecules do use many attempts to overcome the surface barrier before they stabilize on the surface [8, 9].

The goal of this work is to study the adsorption kinetics of proteins on gold and the influence on it of pH and protein concentration in solutions using multi-angle ellipsometry.

2. Materials and methods

2.1. Materials

The horse spleen ferritin (HoSF, type I, Sigma) was qualified for this study because a different character of adsorption processes was expected: unspecific in the case of ferritin and specific for sulfur containing albumin.

To control pH during adsorption process we used both phosphate buffer solution (PBS, 0.05 M KH_2PO_4/$NaHPO_4$) or carbonate one (CBS, 0.05 M $NaHCO_3$) to maintain pH 7.0 and pH 10.0 at 25 ℃, correspondingly. All chemicals used to prepare the buffer solutions are of *p.a.* chemical grade from HANNA Instrument.

The standard cleaning procedure in ultrasonic bath at room temperature was applied to remove organic pollutions on a glass substrate before gold deposition: (a) washing in tetrachloromethane, then (b) in acetone, and, finally, (c) in ethanol. The full process duration was *ca.* 15 min. All the solvents were of electronic grade.

The 12 nm thick gold (Au, N9999, PAMP S.A.) film was deposited onto substrate immediately after cleaning by thermal evaporation from tungsten crucible under vacuum at working pressure of $< 10^{-4}$ Pa. The deposited gold layer has an aperture of 12.7 mm in diameter.

Before each experiment the gold layer was washed with piranha solution (H_2SO_4:H_2O_2 = 3:1, v/v) for 1–2 min, and then with distilled water and ethanol and controlled to ensure that the surface is thoroughly cleaned.

The data were processed and fitted using the special ellipsometric software ELLI3M.

The microphotographs were shot by S-570 (Hitachi) on substrate with absorbed protein after twofold washing in distilled water and drying at 35 ℃ in air.

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2.2. Ellipsometric method

The ellipsometric measurements were carried out on ellipsometer LEPh-3M-1 (Russia) at 632.8 nm using a cell comprising optical glass prism with a semitransparent gold film formed on its hypotenuse face through physical vapor deposition (PVD) process, glass container (Petri cup) and a distance ring (Fig. 1). This arrangement eliminates the protein sedimentation problem. During measurements the container is filled with a protein solution of certain concentration so that the gold surface is immersed into the solution.

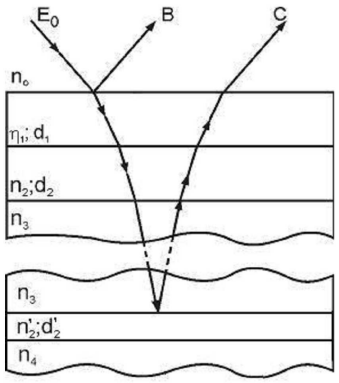

Fig. 1. Scheme of ellipsometric measurement (two-layer model): E_0 — incident laser beam, n_0 — refractive index of prism, $\eta_1 = n_1 - \mathrm{i}\kappa_1$ — complex refractive index of gold layer; n_2, n_2' — effective refractive indices of adsorbed layers; n_3 — refractive index of solution, d_1 — thickness of gold layer; d_2, d_2' — effective thicknesses of adsorbed layers. n_4 — refractive index of glass bottom of the Petri cup.

The laser radiation impinges normally onto the input cathetus face of the prism and then incidents on the hypotenuse face at 45°. This allows to avoid the change in the sounding beam polarization state at the first boundary. On the cell output we have two collinear space-divided beams (B and C) falling at the normal to the second (output) cathetus face of the prism. The front beam B is formed under the reflection from the sub-system "1" (prism–golden layer–adsorbed layer–solution), whereas the rear beam C of much lower intensity (less than 1% of that for beam B) is a result of the reflection from the surface "2" (solution–adsorbed layer–glass).

Onward only the beam B will be taken into the consideration, but the polarization state of the beam C contains the information about adsorption process, as well, and can be used in future setups. In the first approximation we can confine the physical model to consist of two layers: an adsorbent gold layer of constant thickness and adsorbate protein layer whose effective thickness grows due to adsorption of new protein molecules delivered from solution through diffusion. The solution is suggested to be homogeneous and statistically isotropic. The adsorbed layer is assumed to be homogeneous with respect to the distribution of adsorbate structures on the gold surface. Despite the relative simplicity of developed model, it reflects the basic features of the experiment. The principal refractive n_1 and extinction κ_1 indices of the gold substrate depend on the deposition conditions and by this reason they were estimated in separate multi-angle ellipsometric measurements using only buffer solutions.

To describe the changes in the complex amplitudes of two orthogonal linearly polarized waves (p- and s-) reflected from the system, one may use any of numerous methods. We use the well-known 2×2 matrix description to calculate the amplitude reflectance coefficients which define the electric field for the beam B at the boundaries

$$E_{\mathrm{B}}^{p,s} = r_{03}^{p,s} E_0^{p,s}, \tag{1}$$

where E_{B} — the amplitude of the incident laser beam, r_{03} — the amplitude reflective coefficient, E_{B} — amplitude of the reflected beam. All the designations apply to both polarizations s and p.

The angle of incidence in all the experiments was 44.49° for BK7 prism with $n_0 = 1.515$. Usually the ellipsometric data are described using two parameters, ψ and Δ, according to the following expression:

$$r_p/r_s = \tan\psi \exp(\mathrm{i}\,\Delta). \tag{2}$$

For sub-monolayer films and highly reflective surfaces the most sensitive to the adsorbed film thickness parameter is Δ, as ψ changes little. For this reason just that parameter is used to characterize the adsorption of proteins on highly reflective gold surface. The standard deviation for this parameter was estimated as $\sigma_\Delta = 0.13°$.

3. Results and discussion

The measurements of the refractive indices n of the buffer solutions using PR2 refractometer (Carl Zeiss) gave the values depicted in Table. Their extinction indices κ were estimated through spectrophotometric data (Cary 500, Varian) to be less than 10^{-4} at 633 nm. Both indices, n and κ, are practically the same for protein solutions at concentration up to 0.1 mg/ml for ferritin and 5 mg/ml for albumin. Measurements carried out on polarimeter Palomat A (Carl Zeiss) indicate that the optical activity of solutions is negligible (< 10 μrad/mm) and they are statistically isotropic. In computer simulation for gold layer there was used complex refractive index $\eta_1 = n_1 - \mathrm{i}\kappa_1 = 0.198(\pm0.002) - \mathrm{i}3.43(\pm0.02)$. Refractive indices for adsorbed proteins are assumed to be $n_2 = 1.55$.

TABLE

Indices n and κ of the buffer solutions.

No.	Buffer	pH	n	κ
1	0.05 (KH_2PO_4)/($NaHPO_4$)	7.0	1.335±0.002	$< 10^{-4}$
2	0.05 ($NaHCO_3$)	10.0	1.337±0.002	$< 10^{-4}$

The change of the ellipsometric angle Δ versus time for adsorption of ferritin from alkaline solutions (0.01

and 0.1 mg/ml, pH = 10.0) is depicted in Fig. 2. In Fig. 3 there is given a scanning electron microscopy (SEM) image of the substrate after adsorption from 0.1 mg/ml solution for 150 min. The dependence of Δ vs. time reveals unsmooth behavior, which could be explained by episodic changes of both effective refractive index of adsorbate and/or coverage fraction due to the rearrangement of adsorbed protein structures. It seems as if the possible mechanism comprises of the following stages:

— the first one is a fast adsorption of the ferritin molecules at the active sites on gold surface;

— the second stage is a slow growth of linear segments from these occupied sites into the solution volume as they simultaneously grow in width;

— the third one is the change of the orientation of a segment when its length l becomes critical as it relocates in-surface. During this reorientation its structure transforms into more shallow and wider formation.

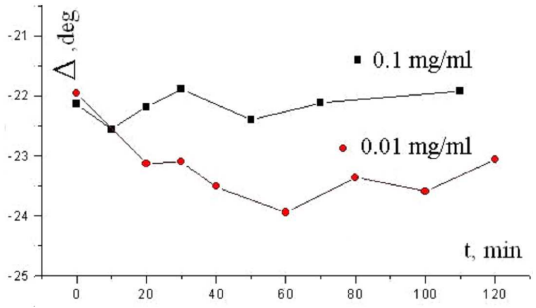

Fig. 2. Change of ellipsometric angle vs. time during adsorption of ferritin at pH 10.0.

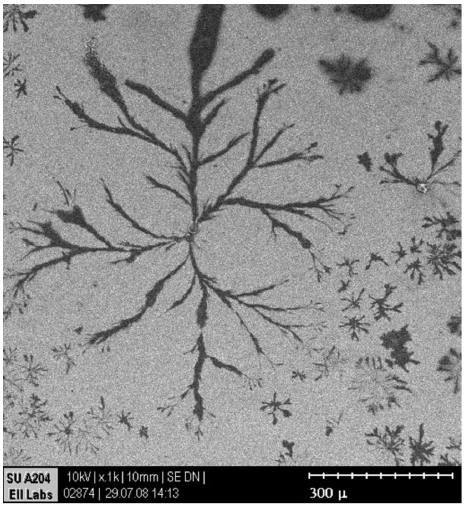

Fig. 3. SEM image of adsorbed ferritin on gold surface from 0.1 mg/ml solution at pH 10.0 during 150 min.

The end of the relocated segment becomes a new site for adsorption and growth proceeds up to new rearrangement, and so on. The vertex formed by two adjacent segments is also a new possible adsorption site. Formed in such a way dendrites are depicted in Fig. 3.

The change of the ellipsometric angle Δ is due to changes in amplitude reflection coefficients of the s- and p-polarized waves at the rear boundary of the golden film and solution and in the first approximation they can be written as

$$r_{03} = r_{01} + t_{01}\,[r_{13}(1-\alpha) + \rho_{12}\alpha]\,t_{10} \times \exp(-\mathrm{i}2\boldsymbol{k}\eta_1\boldsymbol{d}_1). \quad (3)$$

In this expression α (≈ 0.07) is a coverage fraction, ρ_{12} — amplitude reflection coefficient from a sub-system "gold–protein layer–solution", as all the transmission and reflection coefficients have to be indexed for both polarizations (s, p). One can to take into account the interaction of electromagnetic wave with the protein passages of different orientation (in-surface orientation and at angle of 35 ± 5 degree to it, correspondingly), diffraction of sounding light, etc., but these would be excessive in this approximation. The similar experimental results are obtained for adsorption of ferritin at pH 7.0, as well.

The obtained results strongly depend on the experimental conditions, especially the biography of the gold layer, chemical composition of protein, concentration and pH of solution, and others.

The received results show that the adsorption process is definitely influenced by the chemical nature of the protein (specific or non-specific adsorption), and acid–alkaline equilibrium of the medium with the protein. They apparently define biological and physiological behavior of proteins in different pathological processes.

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