LANGMUIR-BLODGETT FILMS OF IMMUNOGLOBULIN AS SENSING ELEMENTS IN MICROGRAVIMETRIC IMMUNOASSAYS

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The work was carried out in the Small-Angle Scattering Laboratory of the Institute of Crystallography in Moscow. The structure of macromolecules and their complexes in solution and in the condensed phase were studied in the same laboratory. Neutron and X-ray small-angle scattering techniques were mainly used for this. One aspect of our investigation was the study of Langmuir-Blodgett films. We also tried to find a practical application of protein LB-films in some biosensor devices.

The key problem in the designing of biosensors is the production of protein films and their subsequent deposition on the surface of sensing elements so as to preserve the native properties of proteins. From all the known methods the Langmuir-Blodgett technique is the most suitable for preparation of ordered monomolecular films and their depositon on substrates. In our case the LB-technique consists of the following: a certain amount of antibodies (antigens) is applied on the surface of a water subphase. Then they are compressed by a barrier to form floating condensed two-dimensional structure on the water-air interface.

Monomolecular antibody LB films were formed on the water/air boundary by Langmuir Trough (Joyce Loebl, England), with a specially made teflon trough. The films were prepared at room temperature, the surface pressure was 20 mN/m [1, 2]. Glutaraldehyde was injected into the subphase (the final concentration was 0.014 M [3]). The glutaraldehyde coupling antibody LB film was transferreed by a "horyzontal lift" onto the both sides of the piezoelectric resonator and after that dried in an argon jet (figure 1). Azometine linkages were reduced during the hours of crystal exposition in 0.02 M sodium borohydride solution. Immunoassay techniques are based on the principle that an antibody will form a complex by selectively binding with the antigen which has stimulated its production.

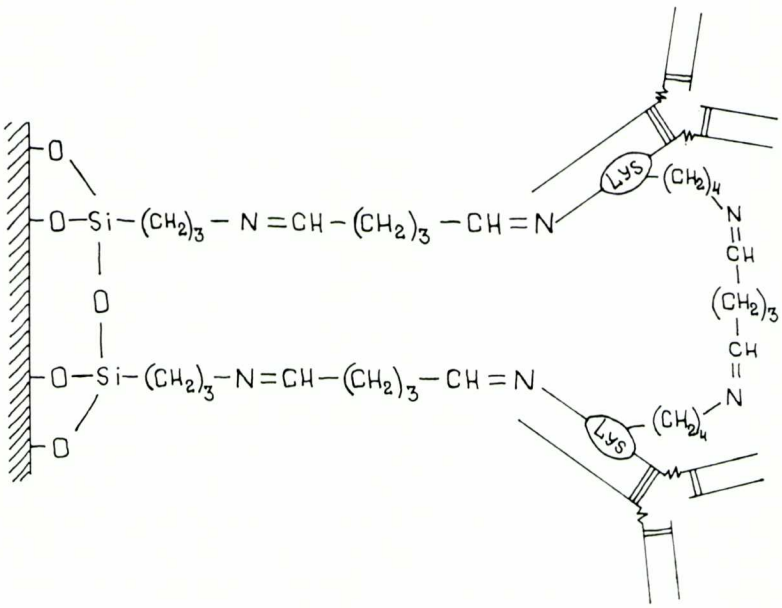

Fig. 1. Scheme of a Langmuir film on a substrate.

The use of piezoelectric sensors for the environment control was described first by King [4]. The application of this method to the analyses of protein media was shown in [5, 6, 7]. A high speed of analysis and no need for antigen premodificatioin are the advantages of the microgravimetric technique as compared with other immunological methods. Microgravimetric method is based on the fact that the surface mass changes caused by adsorption are reflected as shifts in the resonant frequencies of the crystals [8]. The relationship between surface mass changes (Δm) and resonant frequency shift (Δf) is given by the Sauerbrey equation:

$$\Delta f/f = -\Delta m/Arl;$$

where f is the resonant frequency, A is the area covered by the adsorbed material, r is the density of the quartz, l is the thickness of the piezoelectric crystal [8, 9]. Standard 9 MHz quartz crystals with silver-plated electrodes were used. According to the evaluations the binding 0.7×10^{-3} mg of substances corresponds to the frequency shift $\Delta f = 100$ Hz.

The surface is modified by a thin layer of siloxane polymer consisting of 3-aminopropyltriethoxysilane (APTS) in order to attach high reactivity organic groups to the surface of silver electrodes and to protect resonator surface from the influence of water [10]. The resonator surface is first modi-

fied by binding a monomer layer of APTS to the quartz surface. The initial silane layer is polymerized with other silane molecules to form a siloxane polymer with a high reactivity to glutaraldehyde coupling IgG film [11]. In the presence of water, the ester groups APTS hydrolyze to form hydroxyls which react with the silanol groups on the crystal surface. In the absence of water, ester groups condense with the hydroxyls on the surface. The thickness of silane layer is affected by silane concentration, reaction solvent, pH, reaction temperature etc. [12]. According to [1], the shift of resonant frequency as a result of water adsorption from the test solution while modification of its surface by the 5% solution of APTS is about 200 Hz within 20 minutes. The frequency shift is of the same order, depending on specific immunoadsorption.

We used silanization by vapour of APTS in vacuum at the APTS boiling point temperature without any solvent. The proposed method protected the surface of resonator from the influence of solvent (figure 2).

A comparative evaluation of non-specific water adsorption by the piezoelectric crystals modified by APTS in the gas phase and ordinary ones

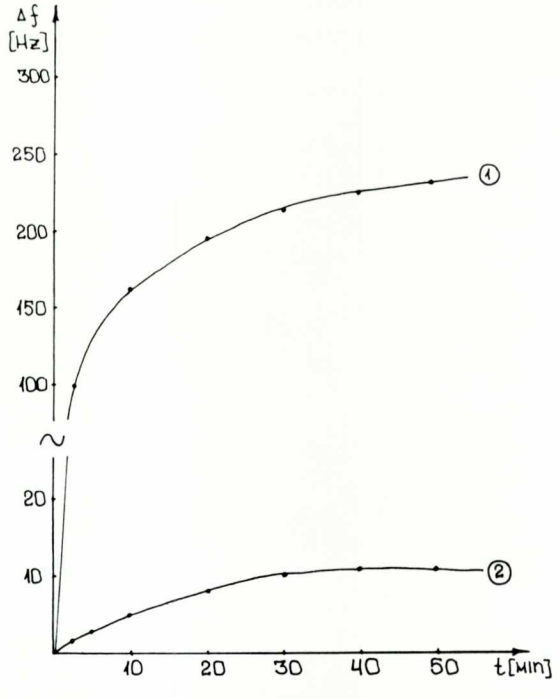

Fig. 2. Non-specific sorption of water by piezoelectric resonators, modified by 3-aminopropyltriethoxysilane in gas phase (2), and by standard resonators (1).

was carried out. The functional dependence of the resonant frequency shift on the time of exposition in water for some resonators is shown in fig. 2. The frequency decrement of a modified APTS crystal is about 20-30 Hz within 30-50 minutes, for ordinary crystal it is about 200-300 Hz within the same time. Therefore non-specific water adsorption may be ignored in further consideration of antibody-antigen binding.

In report experiments, the surface of crystals was covered by LB films of rat antiallotype anti-Igk. 1b antibody. Immunoglobulines Igk. 1b and Igk. 1a differ from one another only in the primary structure of light chains of constant (C) domains. These distinctions are reduced to 11 amino acids changes, from which only two amino acid residues (positions 153 and 155) take part in the formation of the Igk. 1b alloepitope anti-Igk. 1b antibody, pointed to part of the C-domain of light chains. Yet, as stated in [13], these slight amino acids replacements lead to cardinal changes of immunospecificity of immunoglobulines, but do not break the three-dimensional structure of molecules, on the whole.

Microgravimetric measurements were carried out using piezoelectric crystals covered by LB films of anti-Igk. 1b antibodies. Sensors were held in solutions with different concentration of Igk. 1a and Igk. 1b (from 0.05 mg/ml to 0.0005 mg/ml). Figure 3 shows experimental data on the frequency

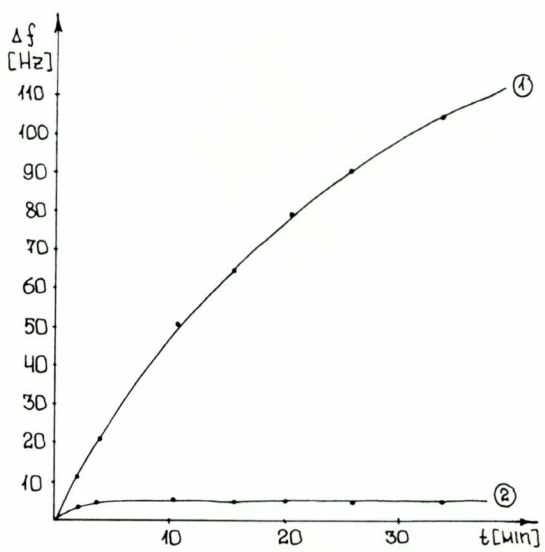

Fig. 3. The frequency shift depending on exposition time of the piezoelectric resonators covered by monomolecular LB-film of rat antiallotype anti-Igk. 1b antibody in test solution. 1.– water solution Igk. 1b (concentration 0.001 mg/ml). 2.– water solution Igk. 1a (concentration 0.001 mg/ml).

shift of the piezoelectric resonators depending on the exposition time in solutions, containing Igk. 1a and Igk. 1b in the concentration of 0.001 mg/ml.

However, considerable concentration (0.05 mg/ml) leads to high binding of proteins of both types, although, as was stated above, Igk. 1a has no specific immunoreactivity to antibodies on the crystal surface. Yet, nonspecific adsorption is negligible, when c = 0.001 mg/ml. The frequency shift is the parameter, which characterizes specific antibody-antigen interaction.

The above described complex of techniques for the standard piezoelectric resonators – silanization in gas phase, deposition of antibody LB films on the crystal surface, microgravimetric measurements – are good when used as sensing elements for detection of the antbody-antigen binding in solution with the antigen concentration in the range from 0.01 mg/ml to 0.0005 mg/ml.

ABSTRACT

Monomolecular Langmuir-Blodgett antibody films were prepared by Langmuir Trough (Joyce-Loebl) under a surface pressure of 20 mN/m at room temperature. Glutaraldehyde coupling LB films were then deposited onto a piezoelectric crystal modified with a thin layer of siloxane polymer carrying a high reactivity amino groups for chemical attachment of a monolayer of antibody and protection of the crystal surface from solvent penetration. The event of antibody-antigen binding was studied by the microgravimetric method, using a mass-sensitive quartz resonator to detect and measure the amount of antigen in a test solution. The detection limit of the developed methods and the range of application of the piezoelectric resonators as sensing elements in the immunoassay are discussed.

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