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Affinity interactions on a liposome surface detected by ultrasound velocimetry

R. Krivanek [a], P. Rybar [a], S. Küpcü [b], U.B. Sleytr [b], T. Hianik [a,*]

[a] *Department of Biophysics and Chemical Physics, Faculty of Mathematics and Physics, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovak Republic*

[b] *Zentrum für Ultrastrukturforschung, Universität für Bodenkultur Wien, Gregor-Mendel-Strasse 33, 1180 Vienna, Austria*

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Abstract

In this work, we performed targeted immobilization of immunoglobulins by means of bacterial S-layer proteins from *Bacillus coagulans* E38-66/V1 recrystallized on liposomes, which were exploited as immobilization matrix for antibody (Ab)-human IgG. The study of interaction of rabbit or swine anti-human IgG as antigens (Ag) was performed by means of measuring changes of ultrasound velocity. We showed that at a temperature of 25 °C, the increment of ultrasound velocity [*u*] linearly decreased following an increase of concentration of Ag. The decrease of [*u*] was presumably due to changes of hydration of the membrane due to the binding process. Approximately 10 times lower changes of [*u*] were observed at 45 °C for Ag–Ab interaction as well as for nonspecific interaction of Ag with liposomes covered by S-layer without Ab. No substantial differences in the behaviour of [*u*] were observed for interactions of human IgG with rabbit or swine anti-human IgG.

Keywords: IgG; S-layer; Liposomes; Hydration; Ultrasound velocimetry; Densitometry

1. Introduction

Among a variety of immunoassays, the methods based on liposomes are of special interest. Liposomes are spherical particles that are spontaneously formed in water, containing one or more lipid bilayers and having size distributions that depend on the method of preparation. During preparation, the proteins that are dissolved in water can be encapsulated into the aqueous interior of the liposomes or may adsorb to the surface (interior or exterior) of lipid membranes. Their surface can be modified by antibodies (Ab), so that the antigen (Ag) can interact with the liposome surface. The Ag–Ab reaction can alter, for example, the permeability of the liposomes and, therefore, can be detected by measuring the amount of encapsulated compound that is released from the liposomes [1]. The disadvantage of this method is the necessity to use fluorescent probes.

Recently, we demonstrated that Ag–Ab interaction on liposome surfaces can be detected by means of ultrasound velocity measurement [2]. For immunochemical reactions on the surfaces, the location and accessibility of immobilized antibodies is of major importance. Recently, a novel strategy for immobilization of functional molecules on closed monolayers was developed by using crystalline bacterial cell surface layers (S-layers) as a matrix. It was shown that S-layers can be recrystallized on lipid film and liposome surfaces leading to much more stable structures than unsupported lipid films [3].

In the present work, we applied the method of ultrasound velocimetry and densitometry to study the properties of Ag–Ab interaction on a liposome surface covered with S-layers from *Bacillus coagulans* E38-66/V1. The immunoglobulin human IgG was immobilized to S-layer proteins. We showed a high sensitivity of ultrasound velocimetry to detect Ag–Ab reactions with a detection limit of around 0.1 nM. Furthermore, this approach does not require any chemical modification of Ag.

2. Experimental

2.1. Sample preparation

Liposomes (diameter ~ 200 nm) composed of dipalmitoylphosphatidylcholin (DPPC), cholesterol (Sigma, St.

* Corresponding author. Tel.: +42-1-2-6029-5683; fax: +42-1-2-6542-6774.

E-mail address: hianik@fmph.uniba.sk (T. Hianik).

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Louis, MO) and hexadecylamine (Fluka, Buchs, Switzerland) (molar ratio 10:1:1) were prepared by the "thin-film" method (see Ref. [4] for more details). The final concentration was adjusted to 13 mM of total lipid, which corresponds to 9.5 mg/ml. *B. coagulans* E38-66/V1, a thermophilic organism, was grown aerobically in continuous culture. S-layer proteins were recrystallized on liposomes according to Ref. [4]. Free carboxyl groups from recrystallized S-layers were activated as described in Ref. [2].

2.2. Ultrasound velocimetry and densitometry

The measurement of ultrasound velocity, u, allows to evaluate elastic properties of aqueous media, e.g. liposome suspensions. This evaluation is based on a simple relationship: $u^2 = 1/(\beta\rho)$, where β is the coefficient of adiabatic compressibility and ρ is the density. In the study of mechanical properties of solutions, measuring a relative change in a physical parameter per unit of solute concentration rather than its absolute value is often more important, precise and easier to interpret [5]. In the analysis of mechanical properties of solution, the specific apparent compressibility φ_K/β_0 is useful:

$$\varphi_K/\beta_0 = -2[u] - 1/\rho_0 + 2\varphi_V \quad (1)$$

where φ_K is the specific molar compressibility, β_0 is the adiabatic compressibility of the solvent, $[u]=(u-u_0)/cu_0$ is the concentration increment of sound velocity (u and u_0 are the sound velocity of solution and solvent, respectively, while c is the variable concentration of a solute), ρ_0 is the density of the solvent, $\varphi_V=[1-(\rho-\rho_0)/c]/\rho_0$ is the specific partial volume of the solution, and ρ is the density of the solution. The values of $[u]$ and φ_V can be determined by sound velocimetry and densitometry [6], respectively.

Ultrasound velocity was measured using a differential fixed-path velocimeter consisting of two acoustic resonators [2,5]. The determination of resonance parameters was based on measuring the resonance frequency, f. This was performed by means of a computer-controlled phase-frequency feedback circuit. The relative changes of sound velocity were determined using the relation $\Delta u/u \approx \Delta f/f$. The sample volume was 0.7 ml. Both resonators were equipped with magnetic stirrers for continuous stirring of the solution during measurements. One resonator contained the liposome suspension while the other was filled with the solvent—10 mM phosphate buffer (pH 7.4)—as the reference. The frequency f_0 of the measuring signal was 7.2 MHz.

For precise density measurements, the vibrating tube principle was used to determine the specific partial volume, φ_V, of the solution [6], employing the densitometer system DMA60 with DMA 602 M (Anton Paar, Graz, Austria). In order to obtain a higher resolution, the measurements were performed with two sample cells DMA 602 M. One contained the sample and the other one the solvent.

2.3. Experimental errors

The uncertainty in the concentration of the phospholipid or protein suspensions was smaller than 0.25%. The temperature of the cells was controlled to within 0.02 °C with Lauda RK 8 CS ultra-thermostat (Lauda, Germany). The accuracy of determination of the sound velocity increment, $[u]$, and the specific partial volume, φ_V, was better then 10^{-3} ml/g. The accuracy of the determination of the density was better then 10^{-3} g/ml. Each series of measurements was performed at least three times.

3. Results and discussion

In experiments, we determined changes of the concentration increment of sound velocity $[u]$ of liposome suspension as a function of concentration of rabbit or swine anti-human IgG. Because our studies were focused on estimating the changes of physical properties of liposomes following interaction with immunoglobulins, the value $[u]$ was calculated with respect to the liposome concentration. The addition of immunoglobulins into the measuring cell resulted in a slight decrease of liposome concentration. This "dilution" effect was taken into account in the determination of liposome concentration and, consequently, in the determination of $[u]$ values.

Fig. 1 shows the changes of the $[u]$ value of liposome suspension as a function of immunoglobulins. The non-specific interaction of rabbit anti-human IgG with liposomes covered by S-layers without human IgG resulted in a slight increase of the $[u]$ value (curve 1). The interaction of immunoglobulins with liposomes modified by human IgG (specific interaction) was performed at two temperatures. It

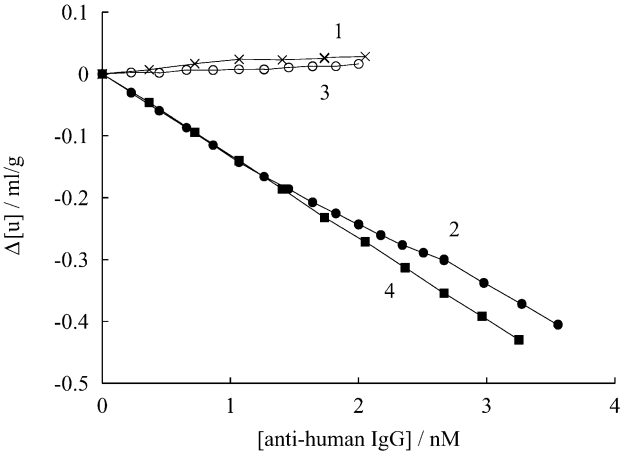

Fig. 1. The concentration increment of ultrasound velocity $[u]$ of S-layer coated liposome suspensions as a function of the concentration of added anti-human IgG. Liposomes without immobilized human IgG, titrated with rabbit anti-human IgG at 25 °C (curve 1). Liposomes with immobilized human IgG, titrated with rabbit anti-human IgG at 25 °C (curve 2) and 45 °C (curve 3), and with swine anti-human IgG at 25 °C (curve 4).

is seen from Fig. 1 (curve 2) that the addition of rabbit anti-human IgG causes a linear decrease of [u] at 25 °C. Considerably different results have been obtained at higher temperature (45 °C). Specific interaction of rabbit anti-human IgG with liposomes resulted in a slight increase of [u] (Fig. 1, curve 3).

Similar results with that for rabbit anti-human IgG have been obtained when swine anti-human IgG was added at 25 °C (curve 4). Slight differences in [u] values have been observed only at concentrations of immunoglobulins higher than 2 nM.

Thus, the results presented above show that specific interaction of immunoglobulins with the liposome surface modified by corresponding Ab depends on temperature and, at 25 °C, resulted in a considerable decrease of [u] value. Rewriting Eq. (1) in terms of the changes of [u], we obtain:

$$\Delta[u] = \Delta\varphi_V - \frac{1}{2}(\varphi_K/\beta_0) \quad (2)$$

We can see that both changes of specific volume as well as changes of specific apparent compressibility contribute to the changes of [u] value. In addition, the compressibility is composed of two terms—compressibility of particles $(\varphi_K/\beta_0)_P$ and that of the hydration shell $(\varphi_K/\beta_0)_H$ surrounding both liposomes and proteins:

$$\varphi_K/\beta_0 = (\varphi_K/\beta_0)_P + (\varphi_K/\beta_0)_H \quad (3)$$

According to Eq. (2), the decrease of [u] value at specific interaction of immunoglobulins with a liposome surface could be explained either by a decrease of specific volume or by an increase of compressibility of liposomes or their hydration shell. The specific volume of liposomes covered by S-layers is approximately 0.924 ml/g (25 °C) and only slightly increases with increasing temperature ($\varphi_V = 0.934$ ml/g at 45 °C). Hence, if the measured changes of [u] would be caused by changes of the volume of the lipid bilayer alone, we would expect a maximal change in volume of 54%, which is obviously not realistic. The specific volume of immunoglobulins is of the order of 0.7 ml/g and does not depend considerably on the kind of immunoglobulins [8]. By means of densitometry, we determined specific volume for IgG: 0.70 ± 01 ml/g (25 °C) and 0.74 ± 0.02 ml/g (45 °C). Hence, if changes of [u] would be caused by changes of specific volume of immunoglobulins alone, we would expect a maximal change of this value of 70%, which is also not realistic.

Considering our previous studies of protein–lipid interactions by means of sound velocimetry (see e.g. Ref. [7]), we cannot expect more than 10% contribution of intrinsic compressibility of proteins and lipid bilayer to the measured value of [u].

One of the possible explanation of the observed decrease of [u] value would be a decrease of hydration of liposomes and proteins following specific interaction. At lower temperatures, e.g. 25 °C, the hydration shell that covers lipid bilayer and proteins is well ordered and its compressibility is lower than that of the unbound water. We suspect that specific interaction of Ag with Ab at the liposome surface is accompanied by aggregation of liposomes. Aggregation of liposomes caused partial loss of the hydration shell. Therefore, due to the additivity of compressibilities, we would expect a decrease of the contribution of the less compressible hydration shell, so the overall compressibility should increase and the [u] value should decrease (see Eq. (2)).

At higher temperature (e.g. 45 °C), the compressibility of the hydration shell is comparable with that of surrounding buffer (see Ref. [5] and references therein). Therefore, despite the fact that affinity interactions take place also at this temperature, we should not expect changes of the [u] value if it is exclusively caused by changes of hydration of liposomes or proteins.

4. Conclusion

Obtained results and their analysis indicate that the observed changes of the value [u] induced by Ag–Ab interaction could be caused by changes of membrane hydration. The sensitivity of [u] value for the detection of the Ag–Ab interaction opens new perspectives for the application of ultrasound velocimetry and S-layers technology for liposome immunoassay.

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