Formation of the layer of influenza A virus M1 matrix protein on lipid membranes at pH 7.0

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The kinetics of interaction of influenza A virus M1 matrix protein with hydrophilic polymer-supported lipid bilayers formed by 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine and 1,2-diphytanoyl-*sn*-glycerophospho-L-serine at pH 7.0 was studied by surface plasmon resonance spectroscopy. The M1 protein was shown to bind to the lipid bilayer almost irreversibly to form a monolayer; this is in line with the key function of the M1 protein, that is, formation of the protein envelope of the virion. An increase in the percentage of negatively charged lipids from 0 to 30% leads to a considerable increase in the protein monolayer density and a several-fold increase in the binding constant (K_a) of the M1 protein with the lipid bilayer up to $(3.60\pm0.11)\cdot10^8$ L mol^{-1}. The negatively charged lipids in the bilayer appear to promote the manifestation of anisotropic properties of the M1 protein, which enable the protein to form reversible non-monolayer structures on the surface. The M1—M1 interaction was found to be reversible and to be characterized by the binding constant $K_a = (6.3\pm0.1)\cdot10^7$ L mol^{-1}.

Keywords: protein adsorption, lipid bilayer, surface plasmon resonance, influenza A virus M1 matrix protein, polymer-supported bilayer membrane.

The M1 matrix protein forms the inner scaffold of influenza A virus; it adjoins the outer lipid membrane of the virus and interacts with ribonucleoprotein (RNP). This protein plays an important role in all key stages of the viral life cycle by providing the mechanical strength of the virion, the exit of the viral genetic material to the cell cytoplasm, and formation of the daughter viral particles.[1] These functions are substantially determined by the acidity of the medium. In a neutral medium, the M1 protein forms the scaffold of a viral particle and binds the genetic material to the lipid envelope. Conversely, in an acidic medium, the matrix scaffold is partly disorganized, the bonds with RNP are destroyed, and, hence, the viral genetic material can escape to the cell cytoplasm.[2—5] The M1 matrix protein, unlike transmembrane proteins, is conserved among various strains of influenza A virus,[6,7] and the conservative epitopes of the M1 polypeptide chain are considered as components for development of a universal anti-influenza drug.[8] This important feature of the M1 protein can be used for the design of universal antiviral agents that would either suppress the formation of or destroy the protein scaffold of the viral particle. Solving this task requires understanding of the mechanisms of interaction of M1 molecules with one another and with the viral membrane at physiological pH.

The M1 matrix protein is a molecule of 27.8 kDa weight consisting of 252 amino acid (aa) residues and formed by three domains: N (2—67 aa), M (91—158 aa), and C (165—252 aa).[9,10] The pH value of the protein isoelectric point, which was found experimentally for 19 influenza A virus strains, is ~8.6.[11] Currently, the crystal structure was determined only for the NM domain of the M1 protein.[9,10,12] The crystal structures of the N-terminal parts of the protein were found to be almost identical at pH 7 and 4.[10,12] Owing to recent small-angle X-ray scattering studies, the structure of the full-size M1 protein in solution at pH 4.7 was elucidated.[13] According to the results, it is a monomer with a clear-cut structural anisotropy consisting of a compact NM-domain 4 nm in diameter and an extended 2 to 9 nm-long weakly ordered C-terminal domain (most often, the length is 6—7 nm). The authors also found that apart from monomers, a small portion of protein clusters occurs in the solution. The structure of the full-size protein in a neutral medium is still unknown. A gel filtration study of a solution of the M1 protein at pH 7.4 led the authors[10] to conclusion that the M1 protein

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2737—2744, November, 2016.
1066-5285/16/6511-2737

exists in a neutral medium as an extended monomer. However, a later study[14] carried out by the same method showed a concentration-dependent oligomerization of the recombinant M1 protein.

The problems faced by the studies of the isolated M1 protein and pronounced discrepancy of the results suggest that functioning of this protein according to its biological role is highly dependent on the microenvironment, which is possibly responsible for the ultimate spatial structure of the protein.

The interaction of the M1 protein with the lipid bilayer attracts attention of many researchers.[15—20] The dual nature of the protein (the presence of positive charge at physiological pH values and a large number of hydrophobic areas in the molecule) provides for various mechanisms for its interaction with the lipid bilayer. In some early studies, the authors concluded that the protein is incorporated into the lipid bilayer [9,16,21,22] and that the preliminary electrostatic interactions are potentially significant for this.[17] However, later studies failed to confirm the conclusion about the considerable incorporation of the M1 protein, although the results pointed to the presence of electrostatic and hydrophobic interactions between the protein and the lipid bilayer.[15,23,24] In some studies, the structue of the protein layer being formed was studied by atomic force microscopy.[19,20,25] However, the differences between the adsorption conditions, the probes used, and statistical approaches to determination of the size of adsorbed M1 protein molecules account for a significant scatter of the results.

The protein adsorption kinetics largely determines the structure of the layer being formed, but attempts to measure the kinetics of the interaction between M1 protein and lipid bilayer have been made so far only in a few works. This was done, most often, by the intramembrane field compensation (IFC) technique[17,19,24—26] and surface plasmon resonance (SPR) spectroscopy.[25,26] The results also led the authors to conclusion that electrostatic interactions are important for adsorption of the M1 protein. The thermodynamic binding constant of the matrix protein to the lipid bilayer in a neutral medium was estimated by the IFC technique. This method is sensitive to the appearance of a charge directly near the lipid bilayer surface; therefore, it may overlook the formation of large non-monolayer structures upon adsorption. Conversely, the SPR method is suitable for detecting objects located at distances up to several nanometers from the surface. Unfortunately, in the above-indicated studies performed by this method, the authors did not succeed in the fabrication of stable lipid monolayers, which can account for the scatter of the results.

Thus, there is no common opinion about the mechanism of formation of the M1 protein layer on lipid membranes and stability of the layer in neutral media. The kinetic parameters of protein—protein and protein—lipid interactions have not yet been determined. The composition of the lipid membrane, the protein concentration, and tendency for polymerization in a neutral medium can considerably affect the formation of the protein layer. All this requires an integrated approach to investigation of this process.

Previously, we studied the kinetics of interaction of the M1 protein with self-assembled thiol layers and demonstrated that the protein binds irreversibly to both a hydrophobic surface[27] and a surface bearing a negative charge due to the presence of carboxy groups.[28] The present study deals with the mechanism of formation of a M1 matrix protein layer on polymer-supported bilayer lipid membranes at pH 7.0. This model system was specially designed for combining the advantages of bilayer lipid membranes and solid-supported membranes, in particular, fluid behavior and stability of the bilayer, the possibility of protein incorporation, and suitability for the use of a variety of experimental methods.[29—33]

Experimental

The following chemicals were used: KCl (chemically pure grade, Reakhim, Russia), 2-(*N*-morpholino)ethanesulfonic acid (MES) (99%, Calbiochem, USA), NaOH (chemically pure grade, Reakhim, Russia), HCl (chemically pure grade, Reakhim, Russia), HEPES (Calbiochem, USA), DMSO (99%, Panreac), propan-2-ol (99.5%, Sigma Aldrich), CHAPS (98%, Sigma Aldrich), bovine serum albumin, BSA (96%, Sigma Aldrich), and lipids: 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) and 1,2-diphytanoyl-*sn*-glycerophospho-L-serine (DPhPS) (both Avanti Polar Lipids, USA).

Isolation and purification of the influenza A virus M1 matrix protein. The M1 matrix protein was isolated from A/Puerto Rico/8/34 influenza (H1N1) virions by the Zhirnov method[34] and characterized by electrophoretic analysis and MALDI mass spectrometry at the A.N. Belozersky Research Institute of Physicochemical Biology (Moscow State University) as described previously.[12,28,35]

Surface plasmon resonance spectroscopy. The adsorption kinetics of molecules was studied by SPR spectroscopy. The experiments were carried out on a Biacore T200 SPR spectrometer (GE Healthcare). In this instrument, the SPR signal intensity is expressed in resonance units (RU). The magnitude of this signal is directly proportional to the surface concentration of the adsorbed compound. According to the instrument manufacturer data,[36] 1 RU corresponds to a protein surface concentration of about 1 pg mm^{-2}.

The fabrication of a polymer-supported planar lipid bilayer. The lipid bilayer was formed by coalescence of liposomes on the surface of the L1 chip (Biacore), which represented a carboxymethylated dextran layer with chemically grafted lipophilic groups.[36,37] The DPhPC zwitter-ionic lipid, which was electrically neutral over a broad pH range, was the main bilayer component.[37] The negative charge of the bilayer surface, similar to the viral membrane charge, was generated by adding DPhPS to the lipid mixture. This choice of lipids was due to the fact that they form virtually defect-free stable reproducible bilayers, which

provided statistically reliable results in the studies of protein adsorption.

For the preparation of a liposome suspension, a solution of lipids in chloroform with an appropriate component ratio was placed on the bottom of a conical microcentrifuge tube and dried to remove the solvent either under argon or in a CentriVap DNA vacuum concentrator (Labconco) at room temperature. Then 500 μL of a buffer solution (100 m*M* KCl, 2 m*M* MES, pH 4.0) was added into the tube and the mixture was stirred with a V-1 plus vortex (Biosan) for 5 min to form a liposome suspension with a lipid concentration of 1 mg mL^{-1}. The unilamellar liposomes were prepared using a LiposoFast-Basic manual extruder (Avestin) with a polycarbonate membrane (pore diameter of 100 nm) placed between two syringes. The liposome suspension was drawn into the first syringe and passed through the membrane into the second syringe. This procedure was repeated 19 times as described earlier.[38,39] The final suspension, which contained virtually uniform-sized monolayer liposomes, was collected from the second syringe, in order to avoid the interaction with the initial suspension.[39,40] The size of the obtained liposomes was checked by dynamic light scattering using a Zetasizer Nano Z analyzer (Malvern).

The lipid bilayer was formed by a modified standard protocol.[36,37] First, the chip surface was prepared for the experiment by two injections of a 2 : 3 (v/v) mixture of propan-2-ol and 50 m*M* NaOH or a detergent solution (20 m*M* CHAPS), each lasting for 30 s, at a flow rate of 30 μL s^{-1}. Then the buffer solution (100 m*M* KCl, 2 m*M* MES, pH 4) was passed through the cell and a freshly prepared liposome suspension was supplied at a flow rate of 2 μL min^{-1} until the SPR signal corresponding to the liposome absorption reached a stationary value (within approximately 60 min). Figure 1 presents a typical kinetics of this process. After that, the attained SPR signal decreased by approximately 200 RU in response to two successive injections of 100 m*M* NaOH lasting for 30 s at a flow rate of 10 μL min^{-1} and alternating with the introduction of a buffer solution. This attests to a low proportion of weakly bound lipid structures. In all cases, the resulting SPR signal corresponded to the criterion of formation of a planar lipid bilayer.[36,41] The subsequent introduction of a 0.1 mg mL^{-1} BSA solution at 10 μL min^{-1} resulted in a slight signal increase (by ~22.5 RU), which was indicative of formation of a virtually defect-free lipid bilayer coating on the chip surface. On the intact surface of the L1 chip, BSA showed high adsorption signals (see Fig. 1, inset), which is in agreement with published data.[36,42,43] The reproducibility of the results was also indicative of high stability of the fabricated lipid bilayers.

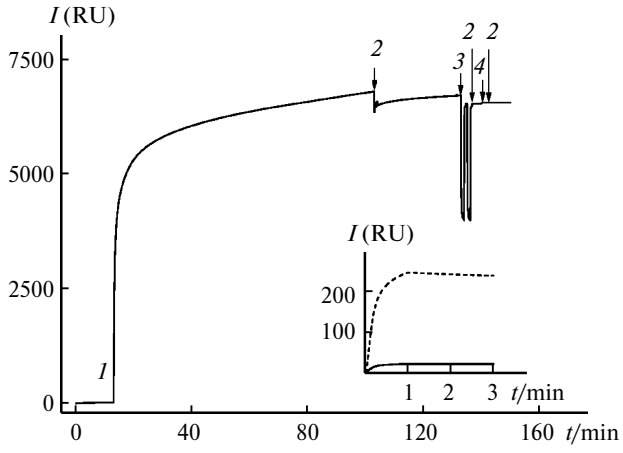

Fig. 1. Formation of a lipid bilayer from a DPhPC and DPhPS mixture in 7 : 3 molar ratio on the L1 sensor chip surface; typical process kinetics: (*1*) injection of a liposome suspension, (*2*) washing with a buffer solution, (*3*) two successive injections of 100 m*M* NaOH, (*4*) injection of a BSA solution in 0.1 mg mL^{-1} concentration. Inset: comparison of BSA binding to the intact surface of the L1 chip (dashed line) and to the lipid bilayer (continuous line).

Study of the interaction of the influenza A virus M1 matrix protein with the lipid bilayer. A typical experimental protocol was as follows: after formation of the lipid bilayer, a protein solution of a definite concentration was passed through the cell at a 2 μL min^{-1} flow rate until the adsorption signal arrived at the plateau. Then the cell was washed with a buffer solution until a new stationary signal level was reached.

For determining the equilibrium binding constants of the M1 protein with the lipid bilayer, the protein adsorption kinetics was measured at various concentrations over a period of 120 s with the subsequent desorption upon washing with buffer solution. This was done using the standard Biacore protocol; for increasing the accuracy of determination of the binding constants according to this protocol, the protein was introduced at high rate, which was 30 μL min^{-1} in the stationary state. For surface regeneration, a 5% DMSO solution was passed through the cell for 30 s. Complete protein desorption and reproducibility of the adsorption kinetic curves served as criteria of the quality of regeneration. The quantitative criterion for reproducibility of the adsorption kinetic curves recommended by instrument manufacturers and used in this study consists in the following: the difference between the SPR signal obtained in a repeated experiment after regeneration and the initial SPR signal must not exceed 10%.

All measurements were carried out at a constant temperature of 25 °C maintained by the instrument thermostat system and repeated 4—6 times.

Results and Discussion

Effect of the M1 concentration on the formation of the adsorption layer. The formation of the M1 protein layer on the lipid membrane consisting of 30% DPhPS and 70% DPhPC was studied at different M1 protein concentrations in solution. The increase in the concentration from 5 to 500 nmol L^{-1} resulted in increasing not only the initial adsorption rate, but also the magnitude of the SPR signal achieved by the 200th minute of adsorption (Fig. 2). An important feature of the kinetic dependences was that the SPR signal did not tend to rapidly reach a stationary level as the concentration increased, but continued to monotonically grow even 200 min after the injection of the M1 protein, despite the high values achieved. This behavior indicated the formation of non-monolayer protein structures; this is in line with the proneness of the M1 protein to aggregation in a neutral medium.[11,13] Therefore, the cell was washed with a buffer solution after 200 min (or later for concentrations of 10 nmol L^{-1} or lower) without

waiting for the adsorption process to be completed. When the protein concentration was 10 nmol L^{-1} or higher, this resulted in a sharp decrease in the SPR signal to a lower level (see Fig. 2); this attested to a weak interaction energy between the removed part of the protein and the surface. It is noteworthy that washing of the cell within 1, 2, 3, or 4 h after protein injection always resulted in a decrease in the SPR signal down to the same level (about 1800 RU). When the protein concentration was 5 n*M*, the SPR signal increased up to the same level, and washing by the buffer solution did not induce noticeable changes. Note that the magnitude of the signal caused by the formation of non-monolayer structures was determined not only by the protein concentration, but also by the flow rate in the cell: as the flow rate increased, the signal decreased. The absence of further desorption upon washing with buffer solution and the lack of dependence of the residual adsorption level (1800 RU) on the protein concentration point to the monomolecular, virtually irreversible adsorption of the protein remaining on the surface. According to the instrument manufacturer data,[35] this SPR signal corresponded to a surface protein concentration of ~1.8 ng mm^{-2}. In the case of the M1 protein, this value corresponds to $3.8 \cdot 10^4$ molecule μm^{-2}. Thus, the surface area per irreversibly adsorbed protein molecule was ~26 nm^2. This is somewhat greater than the unit cell area in the influenza A virus protein network (4×4 nm^2),[15] which indicates the formation of a monomolecular protein layer.

This is a rather typical behavior of proteins, that is, formation of a monolayer strongly attached to the surface on contact with low-concentration solutions and multilayer adsorption (possibly, of aggregates) with increasing concentration of the protein solution.[44—47]

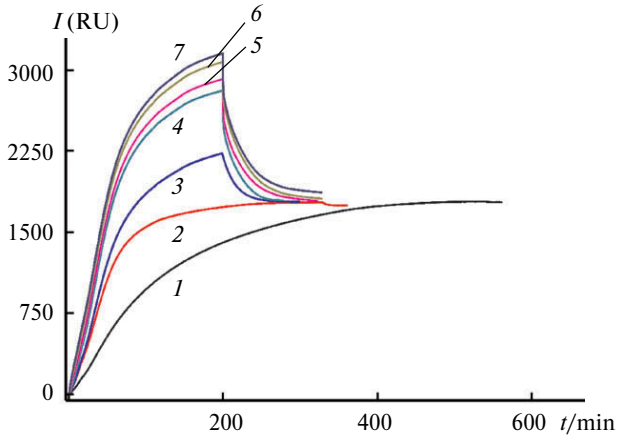

Fig. 2. Adsorption of the M1 protein at various concentrations in solution on the lipid bilayer (DPhPS : DPhPC = 3 : 7) at pH 7.0: protein concentration of 5 (*1*), 10 (*2*), 25 (*3*), 50 (*4*), 100 (*5*), 250 (*6*), and 500 nmol L^{-1} (*7*).
Note. Figures 2, 4—6 are available in full color on the web page of the journal (http://www.linkspringer.com).

In order to confirm the monomolecular structure of the protein layer remaining on the surface after washing with buffer solution, a 4 *M* solution of urea, which loosens the hydrophobic interactions and hydrogen bonds, was injected into the cell during 30 s;[48,49] this was expected to destroy the protein aggregates that could have remained on the surface. Treatment with urea induced only a slight decrease in the SPR signal (by ~60 RU), which confirmed the monomolecular structure of the protein layer formed.

Thus, it follows from the obtained data that, starting with the concentration of 10 nmol L^{-1}, the M1 protein can form non-monolayer structures on the surface, which is in full agreement with the results of our colleagues.[20]

We showed that after a long-term (more than 1 h) washing with buffer solution, a layer of virtually irreversibly adsorbed M1 molecules remains on the lipid bilayer surface. Therefore, we were able to investigate the formation of non-monolayer protein structures by repeated injection of the protein. As can be seen in Fig. 3, this repeated injection of the M1 protein induced a considerable growth of the SPR signal, but the subsequent washing with buffer solution restored the initial signal level. In this case, the M1 protein was bound to the pre-formed protein monolayer; therefore, complete desorption of the added protein points to reversibility of the protein—protein interaction. It is important that, unlike the protein—lipid interaction, this feature is preserved even after a long period of time.

Previously, adsorption of the M1 protein on lipid membranes was demonstrated[24,25] to be irreversible; however, recently, partial desorption of the M1 protein from solid-supported lipid bilayer in a neutral medium was found.[19,20] This provided the conclusion that the protein binding to the lipid bilayer is reversible. Apparently, in the cited studies, the authors have also observed decomposition of non-monolayer protein structures, which we describe here.

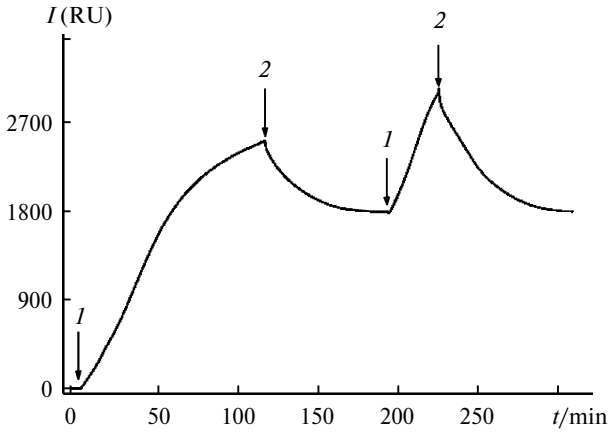

Fig. 3. Partial reversibility of adsorption of the M1 protein on the lipid bilayer (DPhPC : DPhPS = 7 : 3). The arrows mark the time points of injection of the 50 n*M* solution of the M1 protein (*1*) and buffer solution (100 m*M* KCl, 2 m*M* MES, pH 7.0) (*2*).

Effect of the lipid bilayer composition on the M1 adsorption. To determine the effect of electrostatic interaction on the adsorption of positively charged M1 protein, we varied the percentage of DPhPS in the lipid bilayer from 0 to 30% with the protein concentration remaining constant (50 nmol L^{-1}). The dependence of the SPR signal attained by the 200th minute of adsorption (before washing) on the DPhPS percentage is shown in Fig. 4, *a*. It can be seen that the amount of adsorbed protein monotonically increased with increasing lipid bilayer charge and showed tendency to saturation. The adsorption kinetics of the M1 protein was also markedly dependent on the percentage of DPhPS. This is exemplified in Fig. 4, *b* as a comparison of the M1 protein adsorption curves for bilayers containing 0 and 30% DPhPS. As the percentage of DPhPS decreased, the SPR signal corresponding to adsorption substantially declined, reached the stationary level more rapidly, and, after washing of the system, declined to a smaller extent. The final SPR signal corresponding to the monomolecular M1 protein layer on non-charged lipid bilayer was about 20% lower than the same signal for the charged bilayer and was about 1430±50 RU, which corresponds to an approximately 30 nm^2 area per adsorbed M1 molecule. The higher density of the monomolecular protein layer on lipid bilayers containing a negatively charged lipid may be due to their directing force. Under the action of electrostatic attraction, elongated positively charged protein molecules can be arranged predominantly at right angle to the surface. On a non-charged surface without this directing force, protein molecules would occupy a larger area. A similar situation is known for compression of a protein monolayer of elongated globular proteins on a liquid surface.[50] As the surface concentration of the protein increases, the orientation of molecules changes from the ellipsoid longer axis being parallel to the surface to the shorter axis being parallel to the surface.

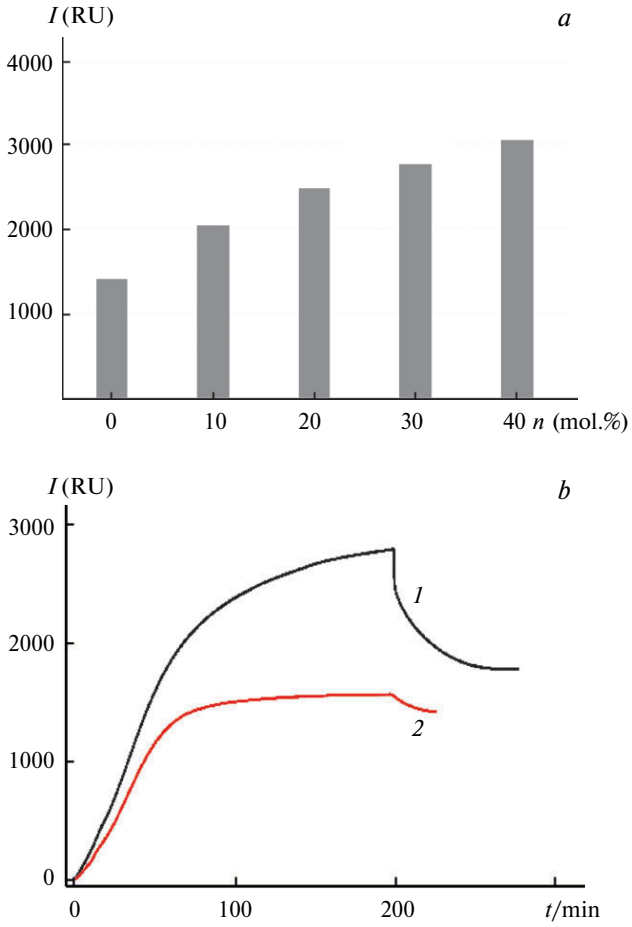

Fig. 4. Effect of the negatively charged DPhPS on adsorption of the M1 protein: SPR signal 200 min after addition of the protein (before washing) *vs.* mole fraction of DPhPS in the bilayer (*a*); comparison of the kinetic curves of adsorption on lipid bilayers containing 30% DPhPS (*1*) and containing no DPhPS (*2*). The M1 concentration is 50 nmol L^{-1} (*b*).

This brings about a question of why non-monolayer protein structures are formed only on negatively charged lipid bilayers. If this was due to adsorption of aggregates able to attach to the oppositely charged lipid bilayer surface, then this would not be observed in the repeated adsorption on the protein monolayer (see Fig. 3). Apparently, upon adsorption on the negatively charged lipid bilayer, the M1 protein exhibits anisotropic properties, promoting the formation of reversible non-monolayer structures. This conclusion is consistent with our assumption about the directing effect of negative charge on the protein molecules during adsorption.

Determination of the binding constants between the M1 protein and the lipid bilayer. The binding constants between the M1 protein and lipid bilayers of various composition were determined by measuring the protein adsorption and desorption kinetics at different concentrations (12.5, 25, 50, and 100 nmol L^{-1}) and at short times where adsorption is expected to be still reversible.[44] The results were analyzed using the Biacore Evaluation Software in terms of the simplest "1 : 1 binding" model:

$$P + S = PS,$$

where P is the M1 protein; S is the lipid bilayer surface.

The M1 protein binding constant was calculated by approximating the kinetic curves assuming uniformity of the constants for all protein concentrations and maximum agreement between the theoretical and experimental data (according to the χ^2 criterion). Figure 5 shows the resulting sensorgrams and the corresponding calculated curves for two lipid bilayer compositions. It can be seen that the model used for approximation describes the kinetic curves rather adequately; this confirms the assumption that the M1 protein adsorption is reversible at short times (up to 120 s). The corresponding adsorption and desorption rate constants(k_a and k_d) and the binding constant of the M1 protein to the surface (K_a) are presented in Table 1.

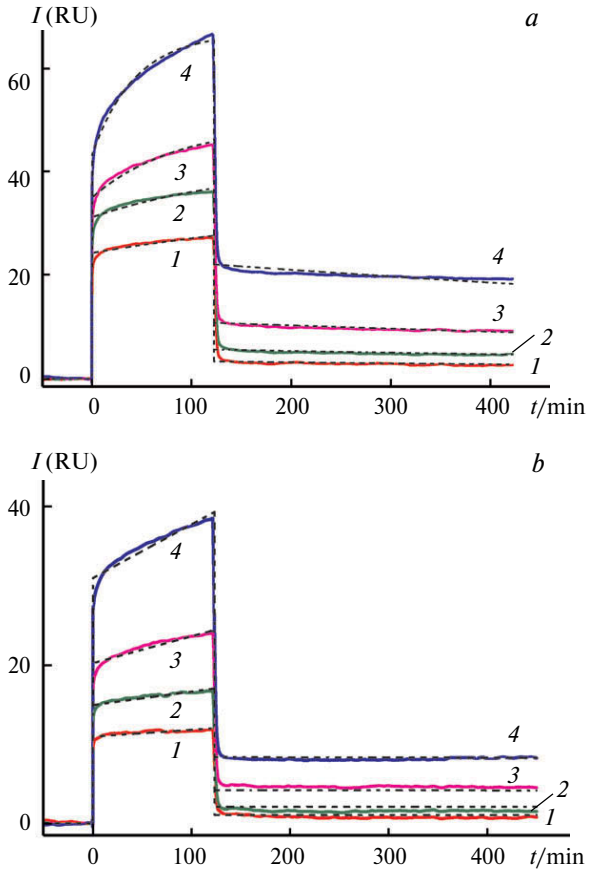

Fig. 5. Determination of the kinetic parameters of interaction between the M1 protein and lipid bilayers of different composition: DPhPS : DPhPC = 3 : 7 (*a*) and 100% DPhPC (*b*). Concentration of the protein solution: 12.5 (*1*), 25 (*2*), 50 (*3*), and 100 nmol L^{-1} (*4*). The dashed curves correspond to calculated dependences.

It follows from the results that increasing the percentage of negatively charged DPhPS to 30% increases the binding constant of the M1 protein approximately 8-fold compared with that for a lipid bilayer containing only DPhPC. This attests to a considerable role of the surface charge and, as shown above, leads to the formation of the adsorption layer of a higher density.

Table 1. Kinetic parameters of the M1—lipid bilayer and M1—M1 interactions

Interaction	Percentage of DPhPS (%)	$k_a \cdot 10^5$ /M^{-1} s^{-1}	$k_d \cdot 10^{-4}$ /s^{-1}	$K_a \cdot 10^8$ /L mol^{-1}
M1—lipid bilayer	30	2.26±0.05	6.20±0.13	3.60±0.11
	0	0.01±0.00	0.27±0.04	0.47±0.07
M1—M1	30	0.83±1.10	13.00±0.06	0.63±0.01

Note. The standard deviation (SD) is given for the values.

An attempt to estimate the protein—lipid interaction constant has been made previously[20] by describing the increase in the attained boundary potential difference with increasing protein concentration by the Langmuir isotherm. The binding constant for the lipid bilayer containing 30% negatively charged lipid in a neutral medium, obtained in this way, was 14 times lower than the value that we found, although in view of the multipont nature of interaction and increasing irreversibility of adsorption with time, the binding constant after completion of the adsorption is expected to be higher. The use of the Langmuir and the Brunauer, Emmett, and Teller theories for describing the adsorption of the M1 protein is not quite legitimate, as they do not take into account the lateral interaction, which is typical of this protein. Moreover, when the molecules are densely arranged in the adsorption layer, their charge is shielded, which may considerably decrease the recorded boundary potential difference. The adsorption of a protein prone to hydrophobic interactions with the Teflon walls of the cell may induce an uncontrolled decrease in its concentration and in the measured signal in dilute solutions; this would give rise to a curve resembling the Langmuir isotherm. This can reduce the apparent constant of protein interaction with the lipid bilayer.

Determination of the effective M1—M1 interaction constant. As shown above, the introduction of the M1 protein in a relatively high concentration in a neutral medium gives rise to non-monolayer protein structures on DPhPS-containing lipid bilayers; these structures are readily destroyed after washing with buffer solution, the irreversibly adsorbed protein monolayer being left on the surface. Determination of the M1 protein binding constant with such a monolayer serves for quantitative characterization of the protein—protein interaction. The corresponding sensograms and the approximating theoretical curves are presented in Fig.6. The binding constant ($(6.3\pm0.1)\cdot10^7$ L mol^{-1}) obtained in this way was ~6 times lower than that for interaction of the protein with the lipid bilayer containing 70% DPhPC and 30% DPhPS (see Table 1). The rate constant for the desorption of M1 from the protein monolayer was two orders of magnitude higher than that for desorption from the non-charged lipid bilayer, which accounts for reversibility of formation of the M1 associates.

The binding constants of the M1 protein with non-charged lipid bilayer and with the M1 protein monolayer proved to be similar in magnitude; however, long-term adsorption revealed a considerable difference between these two types of interaction. The M1 adsorption on the lipid bilayer becomes virtually irreversible with time, whereas protein associates are easily destroyed by washing with buffer solution even 3 h and more after the start of the adsorption (see Fig. 2). Apparently, the M1 protein adsorotion on the lipid bilayer becomes stronger with time owing to the increase in the number of bonds between them.

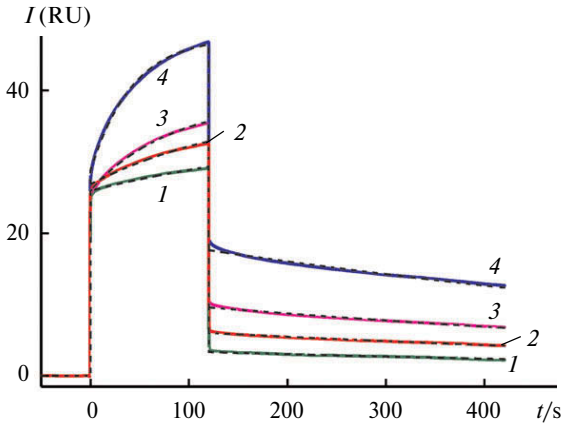

Fig. 6. Determination of the kinetic parameters of interaction of the M1 protein with a preformed monolayer of this protein. Concentration of the protein solution: 12.5 (*1*), 25 (*2*), 50 (*3*), and 100 nmol L^{-1} (*4*). The dashed curves correspond to calculated dependences.

Thus, we created a system simulating the lipid envelope of the influenza A virus and representing a stable almost defect-free lipid bilayer composed of DPhPS and DPhPC mixtures of various molar ratios. In this system, we studied the formation of a layer of the M1 matrix protein at pH 7.0. Study of the M1 protein adsorption and desorption kinetics demonstrated that the protein binds to the lipid bilayer virtually irreversibly and forms a stable monolayer. An increase in the percentage of DPhPS was found to result in a considerable increase in the binding constant between the matrix protein and the lipid membrane and in the higher density of the protein layer formed. The negative charge of the lipid bilayer surface is apparently favorable for orthogonal orientation of M1 molecules and gives rise to anisotropic properties of the resulting layer, owing to which reversible non-monolayer structures can be formed on the surface. The kinetic constants for the interaction between the matrix protein and lipid bilayers of various composition and the effective protein—protein interaction constant were determined.

The results are in line with the natural mission of the M1 matrix protein: to form a strong single-layer scaffold of the viral particle in a neutral medium. In view of the stability of the protein layer formed, we plan to investigate in the future the mechanism of destruction of the matrix scaffold with decreasing pH using the developed model system.

The authors are grateful to O. V. Batishchev for attention to this work.

This work was partially supported by the Russian Foundation for Basic Research (Project No. 16-04-00563).

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Received August 2, 2016;
in revised form September 26, 2016