FINDING THE PH-INDUCED CHANGES IN THE SHAPE OF MATRIX VIRAL PROTEIN M1 BY SPR TECHNIQUE.

Vladimir BREVNOV\textsuperscript{a}, Andrey INDENBOM\textsuperscript{a,b}

\textsuperscript{(a)} Moscow Institute of Physics and Technology (MIPT), Institutskii per., 9, Dolgoprudny, Moscow Region, Russia, 141700

\textsuperscript{(b)} A.N. Frumkin Institute of Physical Chemistry and Electrochemistry RAS, Leninsky pr., 31, Moscow, Russia, 119071, e-mail: a.indenbom@gmail.com

Abstract

The structure of proteins plays a crucial role in their functioning. We have shown how SPR technique allows to determine the shape of proteins and its changes under influence of external conditions. Adsorption of matrix M1 protein of influenza virus on self-assembled monolayer of mercaptohexadecanoic acid was studied by SPR. Acid groups on substrate surface provide its negative charge similar to the charge of lipid membrane of the influenza virus. The protein adsorbs irreversibly and forms saturated monolayer on the substrate surface. It was shown that the process is governed by electrostatic attraction forces. However, the density of the monolayer depends essentially on the conditions of its formation. It was found that in a neutral medium this protein forms monolayers of identical density at any initial protein concentrations in solution. In an acidic medium (pH below 5) the situation differs. The protein forms more dense layers in concentrated solutions and more rarefied layers in dilute solutions. These results suggest that the protein has rather symmetrical globular shape in a neutral medium, and it becomes an oblong molecule in an acidic medium. In concentrated solutions the elongated protein molecules apparently adsorbs mostly orthogonally to the surface forming a thick coat. In dilute solutions, on the contrary, most of the molecules adsorb laterally, forming thinner layers. This approach can be used to analyze shape of other irreversibly adsorbed proteins and nanoparticles.

Keywords: viral matrix protein M1, surface plasmon resonance (SPR)

1. INTRODUCTION

M1 protein of influenza virus forms a scaffold which is adjacent to the inner side of its lipid membrane. In a neutral medium this scaffold protects a viral particle from external influence. Penetration of the virus into a cell by endocytosis and following lowering of pH in an endosome below 5 decomposes protein network and provides output of genetic material of the virion into the host cell [1, 2]. According to the recent data [3, 4] disintegration of a protein capsid in acidic medium occurs together with conformational changes in protein molecules. The aim of the present work is to study the effect of pH on the nature of the interaction of M1 with a surface, simulating a lipid bilayer of the viral envelope by SPR technique. The model system contained self-assembled monolayer of mercaptohexadecanoic acid carrying a negative charge on its surface.

2. EXPERIMENTAL

2.1. Materials

The matrix M1 protein was isolated from intact influenza viruses A/PR/8/34 as described in [5]. Using this method, M1 was isolated by acidic solubilization of influenza virus membrane with nonionic detergent NP40 (Igepal CA630) in MES buffer (50 mM 2Nmorfolinoetansulfonic acid, 100 mM NaCl, pH 4.0), as it was described before [6]. The protein solutions in all experiments contained 100 mM KCL and 2 mM MES.
To study the protein adsorption by SPR technique the self assembled monolayer of the mercaptohexadecanoic acid was applied to the surface of gold-plated glass chips from its 10 mM solution in hexane according to the procedure described in detail in our recent article [6].

2.2. Characterization

Protein adsorption was studied by SPR refractometer “Biosuplar 6” (Mivitec, Germany) in flat two channel flow cell (approximately 1 × 0.5 × 0.1 cm³) as it was described before in work [6]. A peristaltic pump (MasterFlex C/L, Barland Co., USA) was used to change and recirculate water solutions through the cell in an infinite loop. All measurements were performed at room temperature (24 ± 1°C).

3. RESULTS AND DISCUSSION

M1 protein molecule is composed of 252 amino acid residues, which are distributed between three domains: M, N and C. According to the calculation based on the primary structure of the protein (Fig. 1) it is positively charged and the total charge together with charges of its domains increases in acidic medium.

![Fig. 1](image.png)

Fig. 1. A charge of M1 protein and its domains (N, M, C) as function of pH, calculated by the program «Protein calculator v3.4» [7] according to the primary structure of the protein.

Apparently, it leads to the increase of the electrostatic attraction between the proteins and the surface and growth of their adsorption rate at low pH (see Fig. 2).

It is interesting that adsorption signal in the acidic medium usually reached saturation much later than in neutral one, despite the higher initial rate (See Fig. 3). In the first case, a few minutes after introduction of protein in solution rapid growth of the signal was followed by prolonged adsorption stage of a slow increase. We hypothesized that this is due to stretching of the molecule M1 in an acidic medium and disordering of its C-terminal domain. Laying of such molecules with complicated shape on a surface requires considerable time. An elongated shape of the M1 protein molecule in an acidic medium and weakly ordered structure of its C-domain is indicated by numerous literature data [8-10]. As can be seen from Fig. 3, the protein adsorbed irreversibly at any pH values. No significant desorption is observed after washing the system with buffer solution. These results probably are caused by multipoint binding of the protein with the surface.
Charge of the M1 protein decreases with increasing of pH, and at pH above 5 N-domain becomes negatively charged according to our estimations (see Fig. 1). This fact promotes to a more compact globular shape of the protein which has weak tendency to extend along the oppositely charged surfaces and does not require significant time to create the saturated adsorption layer.

**Fig. 2.** Dependence of initial rate of the protein adsorption on pH. The bulk concentration of the protein is equal to 250 nM. The adsorption was measured in relative units (r.u.) of SPR refractometer reply.

**Fig. 3 a)** Kinetics of M1 adsorption at pH = 4 and pH = 7, protein concentration is equal to 250 nM. **b)** Enlarged fragment of the initial part of the plot (a) that shows essential difference of the initial adsorption rates in acidic (upper curve) and neutral (lower curve) media.
A specific feature of the M1 protein adsorption is not only irreversibility, but also a tendency to the formation of saturated monolayer coat. The adsorption layer formed in the solutions of any protein concentration from 50 to 500 nM did not grow after second addition of the protein solution with the same concentration. It is obvious that the formation of the adsorption layer of elongated protein molecules in acidic medium should depend on their concentration in solution. We have found that the medium density of the adsorbed layer (signal of saturated adsorption) formed in dilute and concentrated solutions varied more with increasing of the solution acidity (see Fig. 4).

![Graph](image)

**Fig. 4.** Effect of pH on the magnitude of the adsorption signal achieved at different M1 concentrations of in a solution: 50 nM, 125 nM, 250 nM and 500 nM (indicated in the figure).

In dilute solutions, a rare arrangement of the M1 protein molecules does not prevent their slow laying along the surface. As a result, the surface is occupied completely by relatively small amount of the protein. In concentrated solutions, on the contrary, the competition of the neighboring molecules leads to orientation of their significant part substantially perpendicular to the surface and formation of a denser adsorption layer.

**CONCLUSIONS**

In addition to study of kinetics of M1 interaction with the substrate surface at different pH values the SPR technique has allowed to make a conclusion about the change in the shape of adsorbed molecules. Multipoint binding of the matrix protein M1 with the surface provides its irreversibility in neutral and acidic media. When pH decreases the protein molecule apparently becomes more elongated and acquires a greater number of potential binding sites with the surface due to increase of a positive charge. Such a molecular shape in acidic medium leads to the formation of saturated adsorption layers of different density depending on the initial M1 concentration in the solution, which determines preferential orientation of protein molecules on the substrate surface.
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