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The model of alkylphenol micelles bound to respective antibodies on the solid surface

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ABSTRACT

The model of binding of micelles of nonylphenol molecules to respective antibodies immobilized on the solid surface is proposed. The actual dimensions of micelles obtained from AFM measurements were used in modelling and allowed to predict the shape of the micelle. An account of non-spherical shape of micelles and their simultaneous binding to several antibodies allows estimation of the micelle binding energy.

1. Introduction

The subject of the registration of different toxins in the environment produced either naturally (biotoxins) or as a result of human activities has become very important nowadays. Alkylphenols, for instance nonylphenol, which appear in water resources as a result of biodegradation of commercial alkylphenol ethoxylate surfactants [1] are of particular interest because of their high toxicity and carcinogenic properties. As was found recently, they possess a serious threat, first of all, to fish, and may be to other animals and even to human due to their oestrogen mimicking behavior [2,3].

A simple method of registration of nonylphenol was proposed previously [4]. The method is based upon a combination of the sensitive experimental technique total internal reflection ellipsometry (TIRE) [5,6] and the direct immune assay approach [7], in which the molecules of toxin (i.e., nonylphenol) can be picked up by specific antibodies immobilized on the surface. This attempt was successful and resulted in the registration of nonylphenol in low concentrations down to ppb (part per billion) level [4]; and this value was not the absolute limit but could be substantially and easily improved. The reason is in the anomalously large response of TIRE measurements. Even less sensitive technique of quartz crystal microbalance (QCM) was capable of registration of such concentrations of nonylphenol. The reaction of binding was proved to be highly specific having the association constant in the range of 10^{-6} to 10^{-7} $mol^{-1}\,l^{-1}$, and resulted in the increase in both the adsorbed layer thickness of 23 nm and added mass of 18.3 $\mu g/cm^2$ at saturation for nonylphenol [8]. All these findings led us to the suggestion of the mechanism of binding of large nonylphenol aggregates (micelles) to respective antibodies [4]. Such micelles of amphiphilic molecules of nonylphenol can be formed in aqueous solutions in the course of diluting the initial solution of nonylphenol in alcohols (methanol or ethanol). This phenomenon may be universal and applicable for many other amphiphilic (hydrophobic) chemicals; it was already proved for T-2 mycotoxin [8]. Moreover, the approach of binding of large molecular aggregates to specific antibodies can be used in biosensing for boosting the sensitivity of the affinity sensors.

A simple model of spherical micelle was proposed in [4] and gave reasonable agreement with the results of TIRE and QCM experiments [4]. The calculations also showed that the micelles were larger than the distance between immobilized antibodies, so they could be simultaneously bound not to one but several antibodies. Yet the micelles may not be spherical but have a flatter shape, as illustrated by Fig. 1. The formation of large aggregates and subsequent substantial increase the film roughness as a result of binding nonylphenol molecules was directly confirmed by atomic force microscopy (AFM) [4]. AFM measurements also showed the presence of very large (100–200 nm) aggregates (which may be formed

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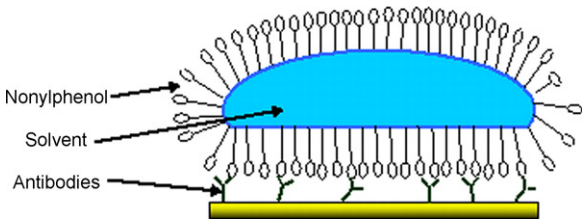

Fig. 1. Sketch of a micelle bound to a surface.

by aggregation of the smaller micelles); and they are definitely not spherical.

The deformation of a micelle gives rise to an additional contribution to the energy of the system—the energy of deformation. The shape of an adhered micelle is then determined by the interplay between the energy of adhesion and the energy of deformation. This suggests that the energy of adhesion of a micelle can be estimated using the experimental data on its geometry.

The influence of the adhesion to the surfaces on the deformation of giant vesicles has been studied previously experimentally [9–13] and theoretically [13–16]. It has been found that bound vesicles can exhibit a large variety of different shapes depending upon the balance between adhesion and deformation energies. A substantial role in this system is played by the thermal fluctuations of the interface which can drive a transition of giant vesicles from a free to a bound state. Gravity can be also important if there is sufficiently large density contrast. On the other hand, the contribution of bending elasticity into the total energy of giant vesicles is usually small, so that bound vesicles typically have a shape of a spherical cap. Moreover, since vesicles comprise a lipid bilayer which typically has small spontaneous curvature [17], the latter can be neglected in the modelling of the system.

The role of these effects is different for bound micelles which have much smaller size and are formed by a monolayer. In this case the role of thermal fluctuations and gravity becomes negligible, while the spontaneous curvature of a lipid monolayer has to be taken into account.

This work is dedicated to a modeling of nonylphenol micelles using the above assumptions. The model may explain the observed experimental features and allows estimation of the binding energy.

2. Experimental details

2.1. Chemicals

10 mM Trizma-base/HCl buffer solution (pH 7.56) was used for the preparation of solutions of all biochemicals except polyelectrolyte; 2 mg/ml solution of poly(allylamine hydrochloride) (PAH); Protein A (0.02 mg/ml) from *Staphylococcus aureus*. All of the above chemicals were obtained from the Sigma–Aldrich catalogue. Antiserum to nonylphenol and nonylphenol were acquired from the Institute of Biochemistry, Kiev, Ukraine. The initial 10 g/ml nonylphenol solution in methanol was diluted with 10 mM Trisma-base/HCl buffer (pH 7.5) to obtain the concentration 20 ng/ml.

2.2. Sample preparation

Samples for the AFM study of nonylphenol binding were prepared as follows. The antiserum to nonylphenol was immobilized on the surface of fresh Cr/Au coated glass slides via the layer of PAH and protein A [4]. Then nonylphenol molecules were specifically bound from its 20 ng/ml solution in Trizma/HCl buffer solution followed by washing out the non-specifically bound nonylphenol molecules in 10% acetonitryl, rinsing in Millipore water, and drying with nitrogen gas. AFM measurements were carried out on two different samples after adsorption of antibodies and after binding nonylphenol:

sample 1: PAH + protein A + antiserum to nonylphenol.
sample 2: PAH + protein A + antiserum to nonylphenol + nonylphenol.

2.3. AFM study

AFM images of adsorbed layers were taken using the NanoScope IIIa instrument operating in tapping mode with the oscillation frequency in the range of 280–310 kHz and the scan rate of about 0.85 Hz. The tip radius was less than 7 nm (probe type was TAP300/RTESP, Veeco). The main focus of the AFM study was on the observation of general features, particle analysis, cross-section and mean roughness of the samples.

3. Model of micelle

In this section we describe a method that allows to estimate the energy of binding of a micelle to a substrate using the data on the diameter of the micelle d and its height h. We shall assume that principal contributions to the energy of a micelle are the adhesion energy E_a, and the deformation energy E_d. For small micelles the entropic contribution to the free energy of the system can be neglected, and the equilibrium shape of the micelle can be derived from the condition for the minimum of the energy which reads:

$$E_a + E_d = \min. \tag{1}$$

We shall use this condition to derive the differential equation which determines the equilibrium shape of the micelle.

We assume the interaction of a micelle with a substrate to be dominated by short range forces (such as specific binding). Therefore, we take into account only the interaction of the amphiphile that is in direct contact with the surface. The details of this interaction can be studied using a statistical model [16]. For our study it is sufficient to describe this interaction in terms of the effective adhesion potential defined as

$$W = -\frac{dE_a}{dS_c}, \tag{2}$$

where S_c is the area of the contact zone of a micelle. For an homogeneous surface the effective adhesion potential W is constant, and the adhesion energy can be written in the form

$$E_a = -WS_c. \tag{3}$$

The deformation energy arises due to finite interface thickness, and is related to the difference of the curvature of a surfactant from the locally preferred (spontaneous) value. We write it in the form

$$E_d = \int_S f_d \, dS, \tag{4}$$

where f_d is the surface density of the curvature energy, and the integral is taken over the surface of the micelle. The widely used form of f_d is given by Helfrich curvature expansion [18]

$$f_d = 2\kappa(H - H_0)^2 + \bar{\kappa}K. \tag{5}$$