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Electrochemical impedance investigation of cholesterol enriched supported films of lipids

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Electrochemical methods of analysis has received a great attention in the enormously large field of Life Sciences at least because of two reasons – first of all, owing to the exclusive role of bioelectrochemical processes at fundamental levels of living matter organization, and second due to specific features of these methods. Some of their advantages as noninvasiveness, sensitivity and inexpensiveness enlarged the reputation of analytical electrochemistry and established its techniques as indispensable tools for research of broad spectrum of problems in Biosciences. Electrochemical Impedance Spectroscopy (EIS) [1-3], on its own, has long been recognized as an extremely informative tool, especially in areas of investigation where contacts between different phases are involved. Biomembranes and their artificial analogs [4, 5] are just such kind of interfaces where the EIS accuracy and sensitivity have already said their heavy word [6]. Most promising results, however, pointing out to a plethora of practical applications, have been obtained with lipid structures deposited on rigid substrates [7, 8]. In the present work we have tried to evaluate the influence of cholesterol on the physicochemical parameters of supported liquid films of lipids, revealed by their specific impedance behavior at different frequencies. The results definitely suggest that cholesterol exert prominent condensing effect on the films, which is demonstrated via the changes in impedance parameters. This is in accordance with commonly accepted role of cholesterol in biological membranes and model systems, which is based on a large body of experimental evidences [9].

Key words: Electrical Impedance Spectroscopy (EIS), lecithin, lipid films, cholesterol.

INTRODUCTION

No doubt, biomembranes, with their morphological diversity, are namely those attributes that give the final shape of the cell and define it as the quant of living matter [10, 11]. Although the native membranes exhibits myriads of forms and functions and their contents comprise thousands of polypeptides, lipids and saccharides, they share a common feature – the canvas of their architecture consists of bimolecular layer of lipid molecules. That cell membranes are arranged over lipid bilayer, became clear after a pioneering work of Gorter and Grendel [12] and in the early 70es Singer and Nicolson introduced their fluid mosaic model explaining how peptides and proteins are attached to it [13]. Furthermore, in the past few decades became evident that lipid bilayer itself is not a passive element of biomembranes, but governs many important cell processes through its physicochemical state [9]. In addition, new concepts for the 3D and 2D (lateral) organization of membranes flourished. Because of their intricate nature biomembranes are often studied via proper models, mimicking their structures and functions. The most popular of these model systems inevitably contain lipid bilayer and for the aim of our survey they could be divided into two major classes: "free standing" [5, 14] and "supported" [15]. Of course, each of them is characterized with their advantages and drawbacks, which are mainly concerned with the great compromise between stability and fluidity [16].

The great importance of the cholesterol in the life is associated with changes that occur in bilayers during the mixing this more peculiar molecule with other lipids. Its extraordinary realization in the biosphere was considered ever since by Bloch as a consequence of changes in the membranes performance in the synthesis of higher sterols.

As a continuation of these ideas, Meyer Bloom, Ole Mouritsen and their collaborators suggested an answer to the question exactly which features of bilayers depend on the cholesterol content [17-20].It has been proved that its ability to give the membranes some very specific physical properties is due to their more flexible behavior, leading to an increase of their structural and functional diversity. Adjusting the phase state of bilayers, cholesterol provides strength and elasticity required for

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reproduction of more complex forms with greater curvature.

In this work we have studied the main effect of cholesterol as revealed by specific changes in the electrical parameters of the films. In this regard, EIS turned out to be reliable method for investigation of the lipid films behavior. The obtained results undoubtedly suggested the ordering role of cholesterol in the films, which is in agreement with the present viewpoint widely described in the literature [9, 21-23].

EXPERIMENTAL

Experimental Set-Up

The experimental set-up used in the work is described in details in [24]. Briefly, the basic part of the set-up is a measuring head containing two identical electrodes forming the electrochemical cell (Fig.1). They are made of gold coated MOS (Si/SiO_2) structures placed on two different holders. The lower holder is machined from brass and contacts a Peltier element, regulating its temperature. The upper holder is moving up and down and its position is controlled by a micrometer, so the distance between the electrodes can be precisely defined.

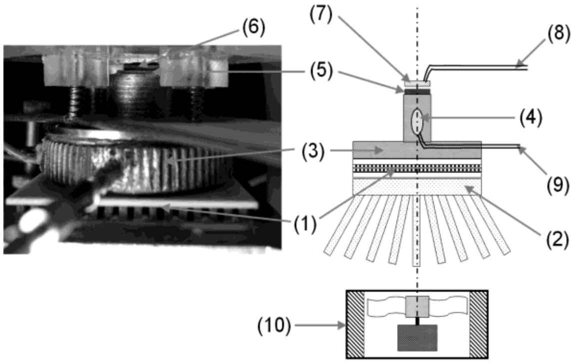

Fig. 1. Overall view (left) and schematic (right) of the experimental set-up: 1) Peltier element; 2) Radiator; 3) Brass holder for the first electrode; 4) Thermoresistor embedded in the brass holder; 5) First electrode - gold (Au); 6) Second Au electrode on Plexiglas holder; 7) Pt thermometer (ceramic substrate with evaporated platinum Pt wire); 8) Leads of Pt thermometer; 9) Leads of thermoresistor; 10) Fan

Materials

Soy-bean lecithin (Walmark, Czech Republic) was used without further purification as lipid material for preparation of the films in this study. Cholesterol (5-cholesten-3β-ol, $C_{27}H_{45}OH$, Sigma Chemical Co. USA) analytical grade, also was not purified additionally. Phospholipid dissolved in n-hexane and sterol dissolved in chloroform/methanol (9/1, vol/vol) were mixed in appropriate quantities to prepare stock solutions with desirable contents.

Gold plated silicon wafers (Microsens SA, Neuchâtel, Switzerland), shown in Fig. 1 were used as solid substrate. The simplest empirical method of preparation of lipid films, known as lipid painting, or paint brush technique, was applied to obtain supported films. Liquid material with different contents of lecithin and cholesterol was deposited directly onto the golden electrode surface, thus so called cast films were realized [25-27].The lipid solution, pre-prepared at the desired concentration, is applied with a micropipette on to the surface of the substrate for each experiments in equal amounts. After evaporation of the on the dry film remained solvent, electrolyte (0,1 M KCl) is added drop wise and it is waited, until hydration of the layer is succeed.

The two electrodes were then gently pressed one to another by a micrometer screw to a desirable distance between them (Fig.1). In some cases Teflon spacer was used to define the distance. An auxiliary "point" platinum electrode is immersed in the in this way formed electrolytic drop. The impedance between this electrode and the gold coating formed lipid film is measured.

RESULTS AND DISCUSSION

Condensingeffect of cholesterol in artificial membranes is a well known and widely investigated phenomenon[28, 29]. For the aim of our survey three types of lecithin films, comprising respectively 49.6, 66.2 and 74.6 mol% cholesterol were studied. First concentration was chosen to represent some higher than normal content of cholesterol in plasmalemma, second one corresponds to saturation limits in phosphatidylcholines and the third is abnormal.

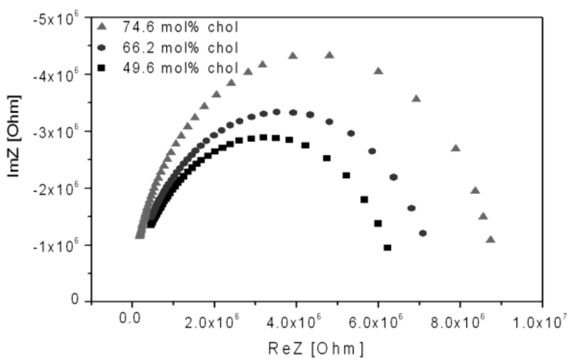

Fig. 2. Nyquist plot for three types of lecitin films with different concentration of cholesterol: 49.6, 66.2 and 74.6 mol%.

The films impedance was measured in the range 130 Hz – 2kHz. Nyquist plots for these frequencies are almost ideal semicircles, revealing increasing modulus of impedance with the increase of the cholesterol concentration(Fig.2). It was useful the impedance to be divided on its components according to the equivalent circuit shown in the inset of Fig. 3.This circuit offered the best fit to the experimental data, obtained with the program ZView. It includes two loops connected in series and represents the closest model, containing minimal number of frequency depending elements (only CPE1).

From the analysis of data, it is clear, that frequency independent parameters R2 and C2 are not very sensitive, while the ARC element, namely R1 and *A*, varies substantially with cholesterol concentration (Fig. 4).

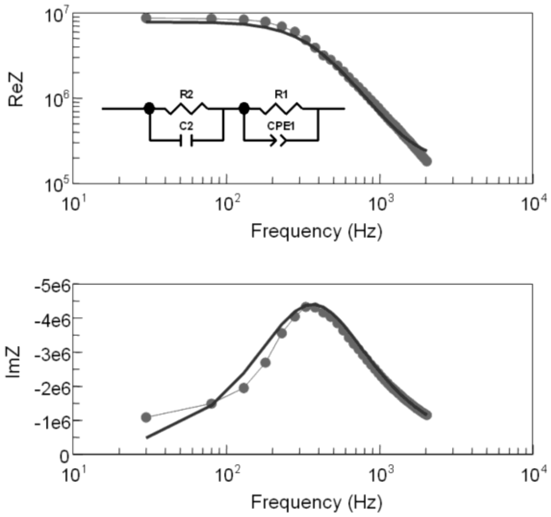

Fig. 3. A fit of the Bode plots with the equivalent circuit used in the work for lipid film with 74,6 mol % cholesterol. A loop of parallel ideal resistor R2 and ideal capacitor C2 is connected in series with so called ARC element consisting of ideal resistor R1 and element with constant phase CPE1. Points are experimental data and line is the fit.

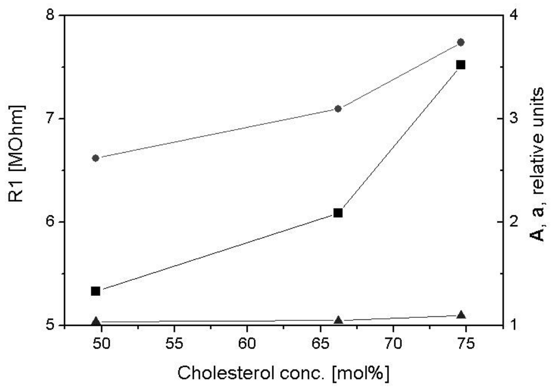

Fig. 4. Depending on the parameters of the equivalent model of the cholesterol content.

Furthermore, the value of ARC parameter *a* is roughly within the limits of unite, indicating that CPE tends to pure capacitor. However a simplification of the model to plain circuit of frequency independent elements would be not very correct and strongly hampered the fitting procedure. Thus, we can speculate, that the discussed films are made up of two peculiar sub areas depending in different way on the presence of cholesterol. Based on earlier studies, we already have built some ideas about them [30]. The one region (or rather both on two electrodes) is the first monomolecular layer adsorbed on the metal surface, plus, eventually, several adjacent layers oriented according to the first. Orderliness of these layers greatly reduced by closely packed molecules in contact with metal, a fully randomly arranged at the transition to the bulk liquid phase, which in fact may be considered a second characteristic region of the film.

Obviously, the mobility (lateral and rotational diffusion)of the molecules in the layer adhered to the substrate is made difficult by the strong adhesive interactions with the solid phase. Typical of this area with reduced mobility is that its impedance Nyquist format is as lightly curved line running in a semicircle for bulk phase [31].The effect of increasing cholesterol concentration is similar, but unfortunately this is not noticeable in Fig.2 because of not so low frequencies (130Hz) of starting the measurements.

CONCLUSIONS

In this work we have used the method of EIS to study the behavior of cholesterol modified solid supported lecithin films. It was found, that such structures are arranged in two distinguished zones – molecular layers more or less closely attached to the rigid support and bulk phase of initial forming solution. Although this general stratification pattern was previously established by investigation of much thinner lipid layers on different substrates, present results shed new lights on the film architecture. Quite certain is that additional profound investigations of the fine structure of the films and of its rising mechanisms are necessary. Moreover they disclosed the abilities of EIS as a reliable tool for investigation of membrane models.

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ЕЛЕКТРОХИМИЧНО ИМПЕДАНСНО ИЗСЛЕДВАНЕ НА ОБОГАТЕНИ С ХОЛЕСТЕРОЛ ПОДДЪРЖАЩИ ЛИПИДНИ ФИЛМИ

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(Резюме)

Електрохимичните методи за анализ получиха значително внимание в изключително обширната област на природните науки най-малко по две причини – преди всичко, благодарение на изключителната роля на биоелектрохимичните процеси на фундаментално ниво при организацията на живата материя и второ, благодарение на някой черти характерни за тези методи. Някои от предимствата им като неинвазивност, чувствителност и изгодност разширяват репутацията на аналитичната електрохимия и установяват нейните техники като незаменими инструменти при изследване на широк спектър от проблеми в биологичните науки. Електрохимичната импедансна спектроскопия (ЕИС) [1-3], сама по себе си, отдавна е призната като един изключително информативен инструмент, особено в иследователски области, където е налице контакт между различни фази. Биомембраните и техните изкуствени аналози [4, 5] са точно такъв вид интерфейси, където с точността и чувствителността си, ЕИС, вече каза своята тежка дума [6]. Най-обещаващи резултати обаче, посочващи множество практически приложения, са получени с липидни структури, отложени върху твърди подложки [7, 8]. В настоящата работа се опитахме да оценим влиянието на холестерола върху физикохимичните параметри на поддържащи течни липидни филми, разпознати благодарение на поведението на техния специфичен импеданс при различни честоти. Резултатите определено показват, че холестеролът оказва основен кондензиращ ефект върху слоевете, което се вижда от промените в импедансните параметри. Това е в съответствие с общоприетата роля на холестерола в биологичните мембрани и моделни системи, основана на голям обем от експериментални доказателства [9].