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Impedance Analysis of Surface-Bound Biomembranes

by

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IMPEDANCE ANALYSIS OF SURFACE-BOUND BIOMEMBRANES

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ABSTRACT

Electrochemical impedance analysis was used to characterize biomembrane structures formed on Si/SiO₂, TiO₂, ITO and Pt electrode surfaces by detergent dialysis. A model equivalent circuit is proposed to describe the membrane/electrode interface. The data suggest that the surface structure is a single membrane layer with resistance of 800 $\Omega \cdot \text{cm}^2$ and capacitance of 550 nF/cm².

INTRODUCTION

We have reported the formation of surface-bound membranes on electrode surfaces by a modified detergent dialysis procedure, which relies upon the self-assembly properties of lipids and membrane proteins [1]. This paper presents an impedance analysis by which the physical structure and electrical properties of the surface-bound membranes are characterized.

RESULTS

Membrane structures containing the visual receptor protein, rhodopsin, were formed by detergent dialysis on silicon (Si/SiO₂), titanium (Ti/TiO₂), indium/tin oxide (ITO) and platinum electrodes that have been "primed" by covalent attachment of long-chained alkyl groups. The electrodes were placed in a flow dialysis chamber with a solution of rhodopsin and photoreceptor membrane lipids in the detergent, octylglucoside. After dialysis against a decreasing concentration gradient of detergent at 10 °C for 20 hrs, the electrodes were removed, washed in buffer and transferred to an electrochemical cell.

The impedance of electrodes with bare surfaces, alkylsilanated surfaces, and surfaces upon which the membrane structures had been assembled were measured in the frequency range of 5 - 100,000 Hz. The electrodes were set at a potential near the open circuit potential for TiO₂, ITO and Pt electrodes. The Si/SiO₂ electrodes were biased such that the underlying silicon was in the accumulation mode. The electrode impedance increased as layers were built upon the surface by the silane reaction, and subsequent formation of the surface-bound membrane.

The impedance of different electrode substrates behaved differently as a function of frequency due to the different electrical properties of the underlying oxide layers. The total capacitance of the membrane electrode is determined by the thickness and the dielectric constants of the oxide, silane and membrane layers. The sensitivity of the measured capacitance to formation of a membrane was particularly influenced by the thickness and dielectric constant of the oxide layer. A larger change in impedance were observed when the membrane was formed on the Pt electrodes than on the others. Figure 1 shows the change of impedance for a surface-bound membrane assembled on a Pt electrode. The increased phase shift in the higher frequency region indicates formation of the low dielectric constant membrane on the surface. The measured resistance of the membrane-coated electrode was 800 $\Omega \cdot \text{cm}^2$.

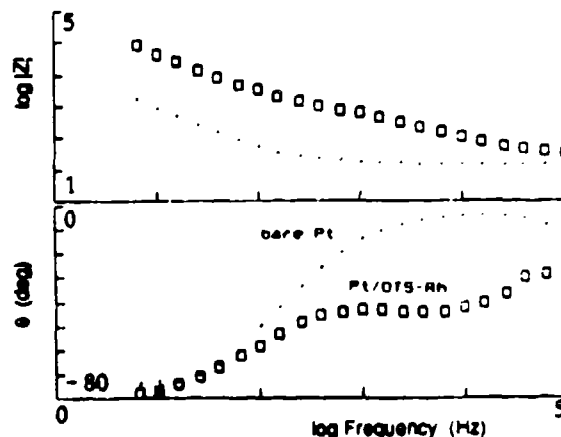


Fig.1. Bode plot for the bare and membrane-coated Pt electrodes.

The experimental data of figure 1 can be modeled by the equivalent circuit shown in figure 2. Cox, Calk, Clip, Cdl and Rox, Ral, Rlip, Rdl are the capacitances and resistances of oxide, alkylsilane, lipid-protein and double layers, respectively. Ru is the electrolyte resistance. The membrane capacitance, Cm, can be represented by a parallel circuit of the tight bilayer capacitance, Cbl (Calk and Clip in series), and the

capacitance of the double layer at defects or pinholes through the membrane. θ is defined as the coverage factor for the tight bilayer on the surface, and $(1-\theta)$ is the fractional area covered by holes.

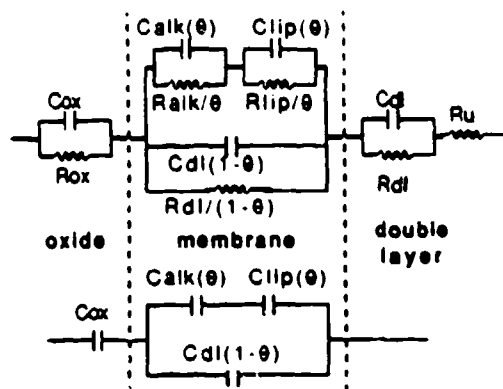


Fig. 2. Proposed equivalent circuit for surface-bound membrane structure.

The theoretical simulation spectrum of this equivalent circuit, shown in figure 3, reproduces the essential features of the experimental data from figure 1. The best curve fit for the coverage factor was 0.97, indicating formation of a relatively complete membrane by the detergent dialysis approach.

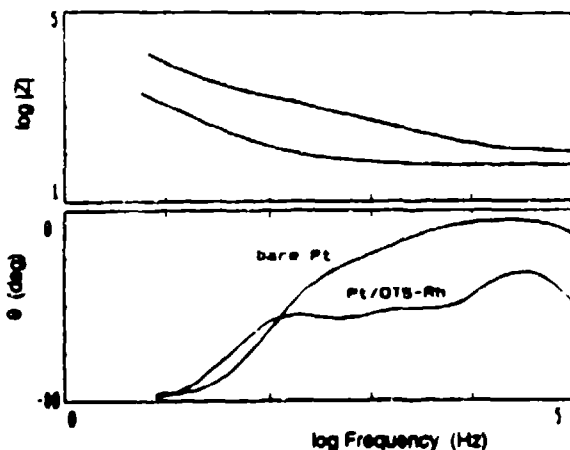


Fig. 3. Simulation of impedance spectrum for proposed equivalent circuit.

Based on impedance data, the Si/SiO₂, TiO₂, ITO and Pt electrodes, with and without the surface-bound membrane, behaved sufficiently capacitively that it may be possible to simplify the equivalent circuit of figure 2. The double layer capacitance is usually 20–40 μf/cm² which is at least

an order of magnitude greater than the membrane capacitance. At higher frequencies, the double layer capacitance acts as a short circuit and can be neglected. The resistances of oxide and membrane layers are usually in the range of 1–100 KΩ. The equivalent circuit can, therefore, be reduced to a single membrane capacitor, C_m, in series with C_{ox}. Thus, $C_m = C_{ox} C_t / (C_t - C_{ox})$, where C_t is the total capacitance of the membrane-coated electrode, and C_{ox} is the measured capacitance of the electrode before membrane formation. The membrane capacitance, C_m, can thus be calculated. Further, the capacitance of the tight bilayer C_{bl}, can be calculated from C_m if the coverage factor and the double layer capacitance are known: $C_{bl} = (C_m - (1-\theta)C_{dl}) / \theta$. The calculated value of C_{bl} was similar for all of these electrodes and averaged about 550 nF/cm², using C_{dl} = 10 μf/cm² and $\theta = 0.97$.

The thickness of the tight bilayer can be calculated by the equation $d = \epsilon \epsilon_0 / C_{bl}$, where ϵ is the dielectric constant of the bilayer. The thickness estimated from the average C_{bl} is 48 Å using a dielectric constant of three. These results are all consistent with a single membrane layer being formed on the electrode surfaces.

CONCLUSIONS

The results from impedance analysis are consistent with a single biomembrane-mimetic structure being assembled on the electrode surface. The structures formed by detergent dialysis may consist of a hydrophobic alkyl layer as one leaflet of a bilayer and the lipid layer as the other. Proteins surrounded by a fairly tight lipid layer may incorporate into holes in the alkyl layer by hydrophobic interactions.

[1] H.G. Smith, J. Li, N.W. Downer & L.W. DeLuca, "Surface-Bound Biomembrane Assemblies", Proc. IEEE/EMBS, Vol. 11, pp. 1329–1330 (1989).

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