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STUDY OF MEMBRANE SURFACE POTENTIAL OF SARCOPLASMIC RETICULUM VESICLES USING pH-SENSITIVE DYE AND PARAMAGNETIC PROBE

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SARKOPLASMAATILISE RETIIKULUMI VESIKULAARMEMBRAANIDE PINNAPOTENTSIAALI UURIMINE pH-INDIKAATORI JA PARAMAGNETILISE SONDIGA. Erika GEIMONEN, Aleksander RUBTSOV

ИССЛЕДОВАНИЕ ПОВЕРХНОСТНОГО ПОТЕНЦИАЛА МЕМБРАН САРКОПЛАЗМАТИЧЕСКОГО РЕТИКУЛУМА С ПОМОЩЬЮ pH-ИНДИКАТОРА И ПАРАМАГНИТНОГО ЗОНДА. Эрика ГЕЙМОНЕН, Александр РУБЦОВ

Key words: Ca-ATPase, sarcoplasmic reticulum, surface potential.

Analysis of electrical properties of biological and model membrane surfaces is one of the new promising approaches to the study of the lipid and protein arrangement in bilayer. Estimation of the influence of different factors on surface potential can give us valuable information about membrane structure changes because protein hydrophilic regions play a key role in the formation of the surface charge, and net surface potential strongly depends on the density and distribution of individual charged groups on the membrane surface.

Sarcoplasmic reticulum (SR) vesicles isolated from rabbit white skeletal muscles were used in this investigation. As it has been shown earlier [1], short-term heating of SR vesicles at 42—45 °C drastically decreases the efficiency of Ca-pump operation without any visible effect on enzyme hydrolytic activity: the so-called uncoupled Ca-pump is obtained in which ATP hydrolysis by Ca-ATPase is not connected with Ca^{2+} accumulation into SR vesicles. It was shown also that the increase of SR membrane permeability for Ca^{2+} was the main reason for thermo-uncoupling [1]. In addition, thermotreatment affects the physico-chemical properties of lipid bilayer that, probably, can change the electrical properties of the membrane surface. Taking this into account we have studied the effects of short-term thermotreatment on the membrane surface potential as well as the influence of bivalent cations on the surface electrical properties of the control and heat-treated SR membranes.

A number of different experimental approaches and tools can be used for studying electrical properties of biological and model membranes, e.g. potential-sensitive spin probes [2, 3], fluorescent probes [4, 5], pH-sensitive dyes [6], and membrane conductance measurements [5, 7].

Quantitative analysis of the effects connected with the electro-chemical properties of membrane surface can be done on the basis of the Gouy-Chapman theory [8]. The relationship between the bulk concentration of a charged species, C_0, the concentration of this charged species next to a charged surface, C_s, and the fixed charge surface potential, Ψ, is given by the Boltzmann equation:

$$C_s = C_0 \exp(-ZF\Psi/RT), \quad (1)$$

where Z is the charge of the species considered, F — the Faraday constant, R — the gas constant, and T — the absolute temperature [8]. The pH value of the solution near the membrane surface (pH_s) differs from pH in the bulk water phase (pH_b), and at the equilibrium at 25 °C these values are connected with the following relationship which has been derived from the Boltzmann equation [9]:

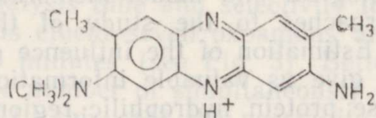
$$pH_s = pH_b + \Psi/60\text{v}. \quad (2)$$

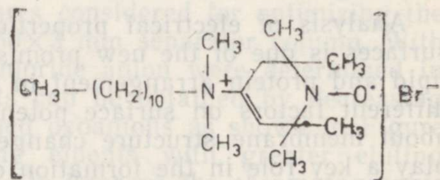
The surface potential of membranes can be studied also with the use of the EPR method by measuring the distribution of charged spin-labelled probes between the membrane and water phases [10]. It has been shown that the distribution of a probe between both phases can be expressed as follows:

$$\lambda = L_b/L_m = \lambda_0 \exp(F\Psi/RT), \quad (3)$$

where L_b is the concentration of the probe in the water phase, L_m — concentration of the probe in the membrane phase, λ_0 — a constant which depends on the chemical potential difference of the two phases [2].

In our investigation we used neutral red as a pH-sensitive dye and the spin probe 4-(*N*,*N*-dimethyl-*N*-decyl)-2,2,6,6-tetramethylpiperidylammonium bromide (CAT10).



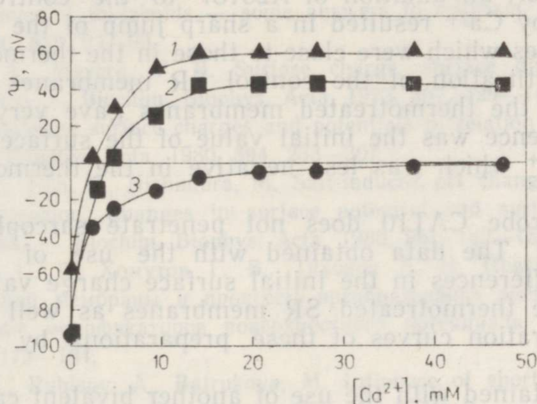


The data obtained show that surface potential is different for the control and thermotreated SR preparations (Table and Figure). As can be seen from the Table, the surface potential of the thermotreated membranes is less negative than that of the control ones. In order to evaluate the contribution of lipids and proteins to the bulk surface potential of SR membranes we have reconstituted Ca-ATPase protein into artificial phospholipid liposomes. A crude preparation of egg lecithin containing mainly phosphatidylcholine and a trace amount of phosphatidylethanolamine was used for reconstitution. The lipid composition of the liposomes was very close to that of SR membranes which contain about 70% phosphatidylcholine, 20% phosphatidylethanolamine, and small amounts of phosphatidylinositol, phosphatidylserine, di- and triglycerides, and cholesterol.

Surface potential values for different membrane preparations *

Preparation	Surface potential Ψ, mV
Intact sarcoplasmic reticulum vesicles	—96.4±4.2
Thermotreated sarcoplasmic reticulum vesicles	—60.2±3.4
Liposomes	0±2.2
Proteoliposomes	—10.1±2.1

* The surface potential has been calculated by Eq. (2) from the pH-measurements with neutral red as a pH-indicator on the membrane surface. The assay medium consisted of 15 mM imidazole, pH 7.0, 5 μM neutral red, 1 mg protein/ml or 0.5 mg lipid/ml at 25 °C.



Effect of Ca^{2+} on the surface potential of sarcoplasmic reticulum vesicles measured by using pH-indicator neutral red.

1 — thermotreated vesicles, *2* — control vesicles with ionophore A23187, *3* — control vesicles. The assay medium consisted of 15 mM imidazole, pH 7.0, 5 μM neutral red, 1 mg protein/ml, 1 μM ionophore A23187/ml, temperature 25 °C.

As can be seen from the Table, artificial liposomes have practically no surface charge. However, incorporation of Ca-ATPase protein into these liposomes leads to the appearance of a negative surface potential. The absolute value of the negative surface potential of the proteoliposomes is about 10-fold lower than that of intact sarcoplasmic reticulum membranes. This seems to be connected with the high lipid-protein ratio in the reconstituted proteoliposomes (6:1 in the proteoliposomes versus 0.5:1 in native SR membranes). Therefore, we suggest that Ca-ATPase protein makes the main contribution to the negative surface potential of SR membranes.

To obtain additional information about the electrostatic properties of the SR membrane surface we have studied the effect of Ca^{2+} and Mg^{2+} ions on their surface potential using control and thermotreated preparations. The Figure shows that comparative titration of these preparations by Ca^{2+} ions gives different results: in the thermotreated SR vesicles the surface charge is changed from negative to positive values whereas in the control ones this parameter is changed from negative to only neutral values.

Since neutral red is located in both the outer and inner monolayers of SR membranes [6], the estimated values of surface charge are the mean values of the potential on both sides of membrane. As the thermotreatment increased the SR membrane permeability for Ca^{2+}, the binding of this ion should take place on both the inner and outer membrane surfaces. This seems to be the main reason for the changes of surface potential from negative to positive values.

For verification of this supposition we have used Ca-ionophore A23187. Since this ionophore drastically increases sarcoplasmic reticulum membrane permeability for Ca^{2+} we could expect some similarities between the effects of Ca^{2+} on surface potential value of the control membranes in the presence of A23187 and on that of thermotreated ones. Indeed, the changes in the surface potential values of the control vesicles in the presence of ionophore during titration by Ca^{2+} were very similar to those obtained with the use of the thermotreated preparations (Figure). In both cases the surface potential changed from negative to positive values. Moreover, an addition of A23187 to the control vesicles after their titration by Ca^{2+} resulted in a sharp jump of the surface potential to positive values which were close to those in the thermotreated preparations. So, the titration of the control SR membranes in the presence of A23187 and the thermotreated membranes gave very similar results. The only difference was the initial value of the surface potential in the absence of Ca^{2+} which was less negative in the thermotreated preparations.

The spin probe CAT10 does not penetrate sarcoplasmic reticulum membranes [10]. The data obtained with the use of this probe also revealed the differences in the initial surface charge values between the control and the thermotreated SR membranes as well as in the character of the titration curves of these preparations by Ca^{2+} (data not shown).

The data obtained with the use of another bivalent cation — Mg^{2+} — were in general very similar to those obtained with the use of Ca^{2+}.

Thus, in our study we first measured the surface potential of SR membranes and investigated the influence of different factors on it. From the data obtained we conclude that Ca-ATPase protein plays the main role in the formation of surface potential in the SR membranes. Thermotreatment of the membranes decreased their surface potential value, probably, via the clusterization of Ca-ATPase molecules [11], and drastically modified the effects of Ca^{2+} and Mg^{2+} ions on the surface potential of the thermotreated SR membranes which may also be connected with the Ca-ATPase protein clusterization as a possible reason for the increase of membrane permeability for these ions.

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