The fluorescent probes application for test of calcium gradient influence on the membrane's structure

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The recent data [1] suggested, that the calcium gradient might be one of the key factors involved in the regulation of the functional states of the Ca^{2+}-ATPase and the Ca^{2+}-channels of Ca^{2+}-store sarcoplasmic reticulum (SR). This study investigates the states of the membrane - bound proteins and lipids and the protein - lipid relationship in depending on the Ca^{2+} concentration gradient on the SR vesicles. The fluorescent probes pyrene and merocyanine 540 were introduced into the vesicles after loading with various concentrations of calcium (0 mM, 0.3 mM, 1mM, and 3 mM). The protein intrinsic tryptophan fluorescence was also used as a probe [2]. Trp fluorescence was applied for the investigations of the organization of the protein molecules and nearest lipids. Pyrene was used for the testing of the physical changes in the lipid phase at the overall bilayer and in the hydrophobic regions at the proteins. For the testing of the transmembrane potential and of the mode of the packing of phospholipids in the outer leaflet of the biological membranes merocyanine 540 was used. Various structural and functional parameters showed biphasic dependence on the Ca^{2+} concentration gradient, with an optimum at 1.0 mM calcium. The main characteristics of the Trp fluorescence for SR - the existence of extreme at the 1 mM of the Ca^{2+}-loading in all our tests. The quenching of the Trp fluorescence by pyrene upon the calcium loading have the minimal extreme at 1mM Ca^{2+}. A similar extreme was observed with pyrene and merocyanine 540. Our experiments with pyrene support the assumption about the decrease in the membrane viscosity, as evidenced by the increase in the fluorescence intensity of the pyrene monomer upon the 1mM Ca^{2+}-gradient. This suggests that the motility of the pyrene monomers may be increased in the more fluid bilayer, and the monomers moved to the phospholipids heads region of the lipid membrane bilayer, where the saturation of the quencher of pyrene emission (oxygen) is smaller. At the poor lipid vesicles, the dependence on the calcium concentration on a manner: 0.3 mM < 1 mM > 3 mM was not being seen. These findings indicate that the main properties of the Trp-containing proteins, the protein-bound lipids and the lipid bilayer upon the 1-mM Ca^{2+} loading are in interdependency with the unique state of SR membrane when all components of the Ca^{2+}-depo exist at the specific orchestration, and when its may be activated immediately at every moment.

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[2] *Vekshin N.L.* // Photonics of biopolimers. Springer "Biological and Medical Physics Series". 2002.