Role of Membrane Dipole Potential-Modifying Agents and Lipid Rafts in Channel-Forming Activity of Syringomycin E

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Comparison of effects of phloretin, an agent decreasing the dipole membrane potential, on ion channels in lipid bilayers containing different sphingolipids, N-stearoyl-phytosphingosine and N-stearoyl-D-erythro-sphinganine, was performed. These sphingolipids have structural analogy with sphingolipids isolated from the wild-type (W303C) and mutant (WΔSYR2α) strains of *Saccharomyces cerevisiae*, respectively. Earlier, the mutant cells were shown to be more resistant to the action of the cyclic lipopeptide syringomycin E (SRE) than the wild strain [1]. The authors of this study conclude that membranes of the wild-type yeast cells with hydroxylated sphingolipids contain lipid domains, rafts, which are lacking in the mutant cell membranes. The authors explain different SRE toxicity for yeast cells of the wild-type and mutant strains by the SRE affinity to rafts. We determined characteristics of the ion channels formed by SRE in bilayer lipid membranes (BLM) bathing in 0.1 M NaCl solutions (pH 6). In the absence of phloretin, current–voltage characteristics of SRE channels, values of their gating charges (Q) as well as ratio of the SRE threshold concentrations required for observation of single channels are equal for the BLM containing 20 mol.% sphingolipids from the wild-type cells and sphingolipids similar to those isolated from the mutant cells. Addition to the membrane-bathing solutions of phloretin at the concentration of 10 μM in the case of BLM containing 20 mol.% sphingolipids from the wild-type cells leads to an approximately 200-fold increase of the SRE channel-forming activity. In the case of the BLM containing sphingolipids similar to those isolated from mutant cells, a more prominent (4500-fold) rise of the steady-state number of the open SRE-channels is observed. It can be suggested that phloretin is unable to change the dipole potential of lipid rafts. Since SRE is mainly concentrated in rafts, addition of phloretin does not produce a considerable increase of the channel-forming activity in the membrane containing sphingolipids of the wild-type cells. In the membranes containing sphingolipids similar to those isolated from mutants, rafts

should not be present; phloretin is uniformly distributed over the membrane thus decreasing its dipole potential and increasing sharply the SRE channel-forming activity. It has been shown in [2, 3] that an increase of the SRE channel-forming activity upon a decrease of the dipole potential of phospholipid bilayers is accompanied by a rise of the channel gating charge. In fact, in the membranes containing sphingolipids of the wild strains, $Q = 2.3 \pm 0.1$, whereas in the membranes containing sphingolipids similar to those present in the mutant strain membranes, $Q = 3.7 \pm 0.5$. As was expected, the increase of the SRE channel-forming activity is accompanied by the rise of the SRE channel gating charge. The obtained data allow the conclusion that in the bilayers containing sphingolipids the SRE channel-forming activity is determined predominantly by the dipole potential of lipid rafts. Thus, it can be claimed that heterogeneity of membranes plays an essential functional role in regulation of ion channels.

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