The effect of cholesterol on alamethicin pore formation in planar bilayers below and above lipid phase transition temperature

S. Überschär*, H. Eibl**, G. Boheim*
* Abt. Zellphysiologie, Ruhr-Univ. Bochum, D-463o Bochum
** MPI Biophysikalische Chemie, D 34oo Göttingen

Using the mixed-chain lipid 1-stearoyl-3-myristoyl-glycero-2-phosphocholine (1,3-SMPC) with a phase transition temperature of T_C = 29°C for the cooling and T_C = 31°C for the heating process virtually solvent-free planar bilayer membranes were formed. The 1,3-SMPC/cholesterol/alamethicin (3 component) system was investigated by single-pore analysis at 2o°C and 4o°C.

Two different kinds of alamethicin pores, short-living (s-) and long-living (l-) pores, are found. Whereas s-pores seem to be the dominating type in cholesterol-free membranes, exclusively l-pores are observed in bilayers with a cholesterol mole fraction $x_{chol} > o.25 - o.3$. Pore state conductances λ_ν are independent of pore type and x_{chol}. Mean pore state lifetimes τ_ν of s-pores are slightly shorter than those of l-pores (within a factor of 2). The most significant effect, however, is the increase of τ_ν with increasing x_{chol} below as well as above T_C. Changing x_{chol} from values $\leq$ o.2 to those $>$ o.2 at 2o°C and from values $\leq$ o.3 to those $>$ o.3 at 4o°C leads to a strong increase of τ_ν of one to two orders of magnitude.

These experimental results are discussed in view of the 'parallel band' model of Owicki and McConnell (Biophys. J. (198o) 3o 383) and the 'percolation' model of Snyder and Freire (Proc. Natl. Acad. Sci. USA (198o) 77, 4o55), respectively, for lipid/cholesterol (2 component) mixtures.

a) Below T_C and $x_{chol} \leq$ o.2 membrane components seem to separate into three phases: 1. a fluid lipid/cholesterol phase of high viscosity with some alamethicin (l-pores), 2. a frozen lipid phase containing only small amounts of cholesterol and alamethicin, and 3. a fluid lipid phase of low viscosity with a small amount of cholesterol into which most of the alamethicin separates (s-pores). A change to $x_{chol} >$ o.2 causes the formation of a continuous lipid/cholesterol phase. Nearly all alamethicin has now accumulated there, because the other domaines vanish, and only l-pores are observed.

b) Above T_C and $x_{chol} \leq$ o.3 membrane components seem to separate into two fluid phases: 1. a lipid/cholesterol phase of high viscosity with some alamethicin (l-pores) and a lipid phase of low viscosity containing a small amount of cholesterol and most of the alamethicin (s-pores). A change to $x_{chol} >$ o.3 leads to the assembly of a continuous lipid/cholesterol phase containing the alamethicin (l-pores).