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Conjunction of alternative mechanisms of gramicidin S embedding into model lipid membranes

R.Ye. Brodskii[1], O.V. Vashchenko[2]

[1] *Institute for Single Crystals of the NAS of Ukraine 60 Nauky Ave, 61072 Kharkiv, Ukraine* r.brodskii@gmail.com

[2] *Institute for Scintillation Materials of the NAS of Ukraine 60 Nauky Ave, 61072 Kharkiv, Ukraine* olga_v@isma.kharkov.ua

Background

- **Gramicidin S (GS) is a prospective antimicrobial peptide which maintains its effectiveness over 80 years since its discovery [1].**
- **Antimicrobial action of GS is directly related to damages of lipid membranes of bacterial cells and related elevation in its non-specific permeability leading to cell lysis and death [2].**
- **The detailed mechanism of GS action is still under consideration. In particular, there are no direct evidences about canonically accepted mechanism of pore formation.**
- **Associate formation was established for GS inside lipid membrane. GS associates are embed into hydrophobic membrane medium, whereas GS monomers locate near hydrophilic membrane surface.**

Objectives

To summarize and agree some literature and our own experimental data within the framework of the proposed theory of mutual impact of alternative mechanisms of a dopant embedding in membrane medium

Materials and methods

- **Differential scanning calorimetry (DSC) of model lipid membranes**
- **Molecular visualization**
- **Mathematical modelling**

Experimental

DSC data suggests that GS interacts with lipid membrane like two different substances (Fig. 1). Conjunction of alternative mechanisms of GS - membrane interaction was supposed to explain a number of features of GS membranotropic action.

GS - membrane system was considered as two-dimension nano-emulsion composed of two different types of lipid domains (Fig. 2) where processes on the domains boundaries, including generation of free volume, impact significantly on the system properties.

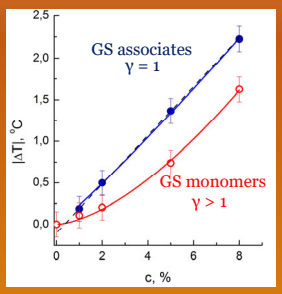

Fig. 1. *Absolute shifts of membrane melting temperature under GS introduction into model dipalmitoyl-phosphatidylcholine (DPPC) membrane*

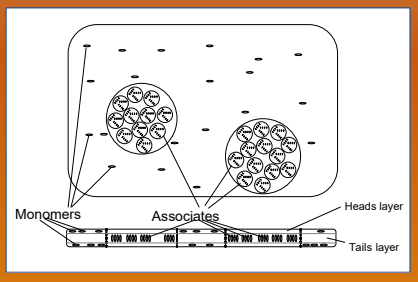

Fig. 2. *A scheme of GS monomers and associates location in the lipid membrane. The upper figure depicts the top view; the lower figure is the side view (parallel to the membrane surface)*

Mathematical modelling of the conjunction

$|\Delta T| = bc^{\gamma}$ if $|\Delta T| \sim s$ (Freundlich adsoption equation)
$|\Delta T|$ is absolute shift of membrane melting temperature
c is dopant concentration in the system
s is concentration of dopant bonded to membrane
b and γ are phenomenological constants (canonically, γ = 0,2 ÷ 0,7)

$s = A_s c^{\alpha} + p_1 A_s c^{\alpha} A_s c^{\beta}$,
where p_1 describes changes in a dopant sorption due to sorption of another dopant
$\Delta T = C_{T1} \cdot c^{\alpha} + C_{T2} \cdot c^{\beta} + C_{T12} \cdot c^{\alpha+\beta}$ – result of conjunction of the two mechanisms

$\Delta T \approx C_{T12} \cdot c^{\alpha+\beta}$ – the term with γ > 1 (see Fig. 1) is determining when separate sorption of monomers and associates is sufficiently lower than joint one

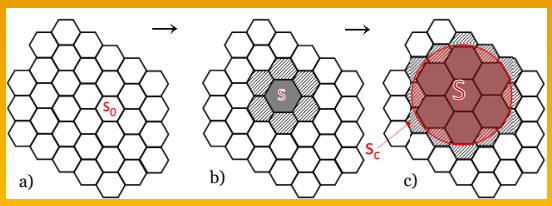

Fig. 3. *A scheme of growth of GS associates-containing domains: a – lipid cells without dopants ('free'); b – a lipid cell embedded with a dopant ('filled') and a layer of adjacent cells with disturbed properties ('disturbed'); c – dopant embedding involves further lipid cells.*

The process of GSA growth (Fig. 3) could distribute involving further lipid cells until accessible free volume will over. So, limitation on GS associate growth is supposed (Fig. 4)

Molecular visualization (MM2) for GS dimer formation suggests the possibility of tight packing of GS molecules without pore formation and any internal restriction to GS associate growth

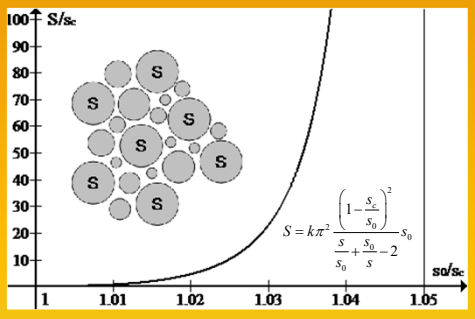

Fig. 4. *Growth of GS associate depending on relation s_0/s_c for $s = 1.05s_c$ and $k = 1$. The insert depicts distribution of GS associates by size*

Model predictions

- **Increase of membrane permeation by mechanism of heterogeneity elevation [4] due to membrane emulsification as main mechanism of GS antimicrobial action**
- **Growth of GS associates only restricts by free volume of membrane**
- **Increase of the maximal size of GS associate with increase of membrane free volume fraction**
- **Forming characteristic sub-clusters (lipid shells of GS monomers and associates) of the order of ten to hundred particles**

Matching experimental observations

- **Increasing in membrane permeability without pore formations**
- **Concentration threshold for registration of GS associates formation**
- **Elevation of the fraction of GS molecules involved in associate formation with membrane thinning**
- **Higher GS affinity to the membranes with lower lipid ordering**
- **Facilitation of GS associates formation in the region of membrane phase transition**
- **Decrease in lipid cooperativity**

Conclusions

- **GS is able to interact with lipid membranes as two different dopants.**
- **A mathematical model of conjunction of alternative mechanisms of GS – membrane interaction was developed and successfully applicated to explain a number of features of GS membranotropic action, including the main mechanism of GS antimicrobial action.**
- **The model could be useful for interpretation and prediction of GS effect in various model and cell membranes.**

References

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