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Morphology of composite hBest1/POPC and hBest1/sphingomyelin monolayers

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Introduction

Human bestrophin-1 (hBest1) is a transmembrane Ca^{2+} - dependent anion channel, associated with the transport of Cl^{-}, HCO^{3-} ions, γ-aminobutiric acid (GABA), glutamate (Glu), regulation of cell volume and retinal homeostasis. Mutations in the corresponding gene lead to retinal degenerative diseases, defined as Bestrophinopathies, but recent research suggests that hBest1 is also connected to neurodegenerative pathologies, such as Alzheimer's and Parkinson's diseases. The lipid environment is essential for the proper function and regulation of hBest1. Using Brewster Angle Microscopy (BAM) we are able to observe differences in the morphology of composite hBest1 / 1 – palmitoyl – 2 - oleoyl – sn – glycero -3 - phosphocholine (POPC) or hBest1 / sphingomyelin (SM) monolayers.

Materials and Methods

The morphology of the monolayers was observed using Brewster's Angle Microscopy (BAM). The quantities of the lipids and hBest1 applied on the interface were calculated so that the ratio of the total surface of the lipid molecules and the total surface of the protein molecules is 3:1. The aqueous phase consists of 150 mM NaCl with one of the following additions: 0.5 μM Ca^{2+}, 2 mM glutamate (Glu) or 100 μM γ-aminobutirate (GABA). The quantitative ratio between the hBest1 and the POPC in the composite monolayer was 1:45 and the same for the hBest/SM monolayer was 1:86. The monolayers were observed in both relaxed and compressed state at a constant temperature.

Results

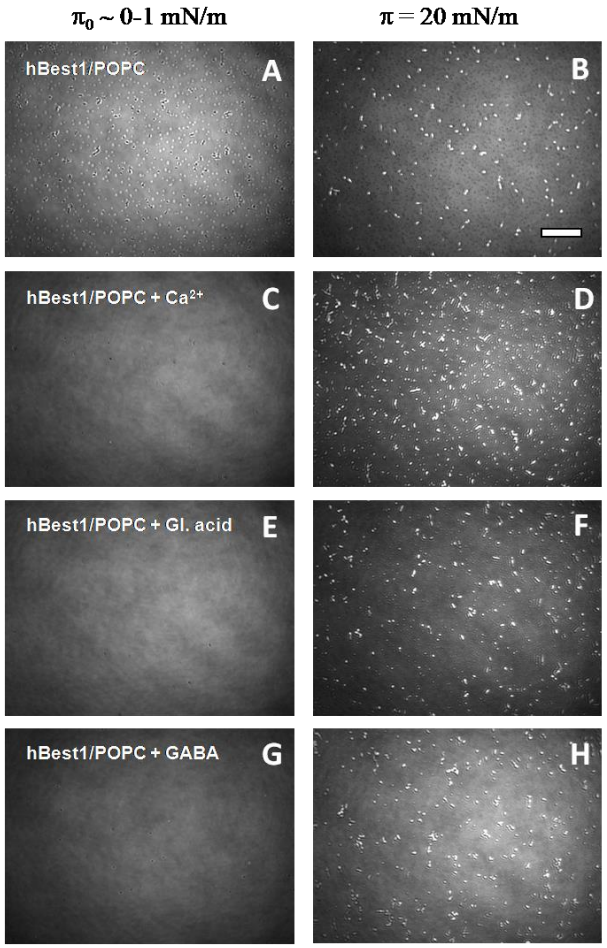

Fig 1. BAM images of uncompressed (left) and compressed at 20 mN/m (right) composite hBest1/POPC monolayers (molar ratio 1/45). The aqueous phase is 150 mM NaCl (A, B) with 0.5 μM Ca^{2+} (C, D), 2 mM Glu (E, F) and 100 μM GABA (G, H). The white scale bar corresponds to 100 μm.

The composite hBest1/POPC monolayers with added Ca^{2+}, Glu and GABA are completely homogenous in relaxed state. In the monolayer on the 150 mM NaCl aqueous phase are observed numerous small denser domains, dispersed evenly in the surrounding homogenous film. Upon compression, the state of the monolayer is similar in all binary systems – homogenous film with evenly dispersed dense domains. The quantity of such domains is the highest with the addition of Ca^{2+} in the aqueous phase. As for the hBest1/SM monolayers, on the 150 mM NaCl the films are homogenous at both compressed and relaxed state. The addition of GABA doesn't influence the monolayer morphology. When Ca^{2+} is added, small dense domains are visible in relaxed state that vanish upon compression. Small domains are also formed when Glu is added in the aqueous phase, but their count and size are considerably smaller than the ones formed when Ca^{2+} is in the aqueous phase.

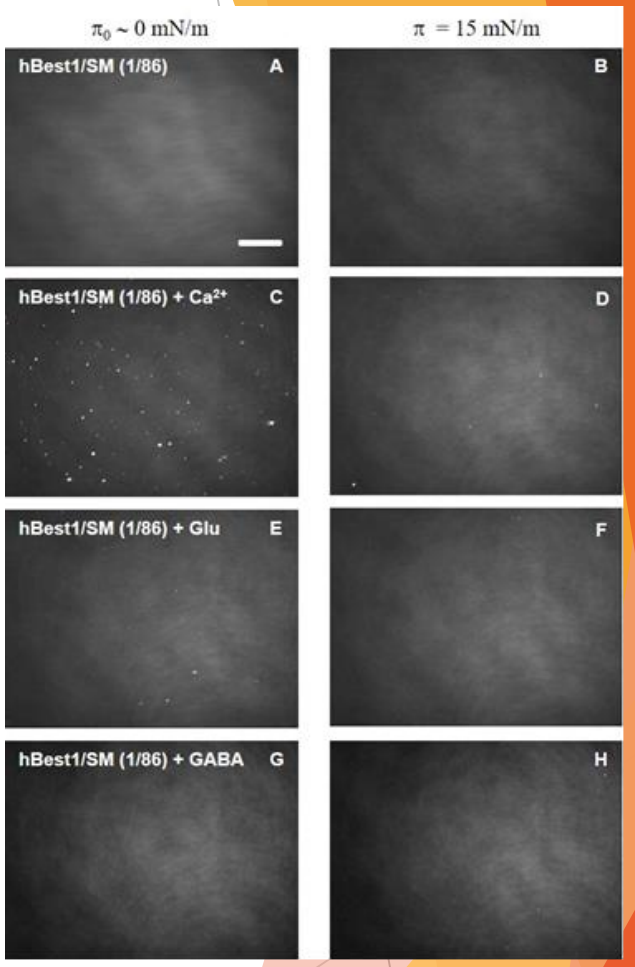

Fig. 2 BAM images of uncompressed (left) and compressed at 15 mN/m (right) composite hBest1/SM monolayers (molar ratio 1/86). The aqueous phase is 150 mM NaCl (A, B) with 0.5 μM Ca^{2+} (C, D), 2 mM Glu (E, F) and 100 μM GABA (G, H). The white scale bar corresponds to 100 μm.

Conclusion

The formation of small dense domains upon compression shows the differences in the interactions between hBest1 and one of the most common phoshpolipids in the cell membrane – POPC and sphingomyelin. The addition of Ca^{2+} in the aqueous phase changes the morphology of the monolayer considerably in both hBest1/POPC and hBest1/SM systems in relaxed and in compressed state, which could be explained by the hBest1 dependency on Ca^{2+}. The addition of Glu or GABA in the aqueous phase shows also morphology changes in the monolayer as opposed to the 150 mM NaCl only aqueous phase, but their effect is visibly less significant in comparison with the presence of Ca^{2+}.

Acknowledgements

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References

- Effects of Ca2+, Glu and GABA on hBest1 and composite hBest1/POPC surface films. T. D. Andreeva, S. D. Petrova, K. Mladenova, V. Moskova-Doumanova, T. Topouzova-Hristova, Y. Petseva, N. Mladenov, K. Balashev, Z. Lalchev, J. A. Doumanov. Colloids and Surfaces B: Biointerfaces 161 (2018) 192–199
- Miscibility of hBest1 and sphingomyelin in surface films – A prerequisite for interaction with membrane domains. N. Mladenova, S. D. Petrova, K. Mladenova, D. Bozhinova, V. Moskova-Doumanova, T. Topouzova-Hristova, P. Videv, R.Veleva, A. Kostadinova, G. Staneva, T. D. Andreeva, J. A. Doumanov. Colloids and Surfaces B: Biointerfaces 189 (2020) 110893