How to determine the surface exclusion pressure of a molecule

INTRODUCTION

Lipid-molecule interactions are of crucial importance in many physiological and industrial processes. To better understand these mechanisms and quantify the affinity between a molecule and a lipid monolayer, its exclusion pressure is often determined [1-4].
Π_e corresponds to the surface pressure above which a molecule can no longer insert itself at an interface. The surface pressure Π is defined by: $\Pi = \gamma_o - \gamma$
where γ_o represents the interfacial tension between two pure phases and γ the measured tension.
According to D. Small's research group, Π_e measures the ability of a peptide or protein to penetrate the polar heads and the aliphatic chains of a phospholipid monolayer [3].
Π_e has been measured for a large number of molecules of interest at interfaces populated by lipids, phospholipids (Table 1).
It is even possible to calculate this parameter for the same protein but at interfaces of various compositions. This is the case of GLTP (glycolipid transfer protein) whose Π_e values vary with the charge of the phospholipids forming the monolayer [5]. The charge of the sub-phase can also significantly impact the affinity of a protein for an interface as is the case for the neuropeptide Y [6].

METHODOLOGY

Briefly, a drop of triolein of 20 µL is formed at the end of a J-cannula immersed in a buffered solution (Hepes 20 mM pH7 NaCl, 150 mM). The oil/water interface displays an interfacial tension γ_(o⁄w)=32 mN⁄m. A solution of liposomes (27.2 µL, 100 nm, 0.5 mg/L) is added to the buffered solution. Phospholipids gradually adsorb at the oil/water interface, reducing the interfacial tension to values between 20-25 mN/m after 1 hour. The aqueous phase is replaced by a fresh buffered solution in order to remove the non-adsorbed phospholipids. The volume of the drop is increased or decreased by the Tracker™ drop tensiometer to reach a desired surface tension γi. This results in an interface with a surface pressure of Π_i :

$$\Pi_i = \gamma_{o/w} - \gamma_i$$

Then a solution containing the protein of interest is injected. Additional lowering of surface tension to a value of γ_{eq} caused by the protein leads to a new surface pressure noted Π_{eq} :

$$\Pi_{eq} = \gamma_{o/W} - \gamma_{eq}$$

The increase in surface pressure $\Delta\Pi$ resulting from the injection of the protein can be written as:

$$\Delta\Pi = \Pi_{eq} - \Pi_i$$

When the addition of the protein doesn't induce any surface pressure increase, that is to say that $\Delta\Pi$ =0 mN⁄m, this means that the surface pressure Π_i is too high for the protein to adsorb at the interface. When $\Delta\Pi$ =0 , Π_{eq} =Π_i.
The experiment is repeated at different Π_i.

RESULTS

For each surface pressure Π_i, corresponding to a given surface concentration of phospholipids, a solution containing the protein to be studied is injected. The addition of the protein causes a decrease in interfacial tension, reflecting its adsorption as illustrated in Figure 1.

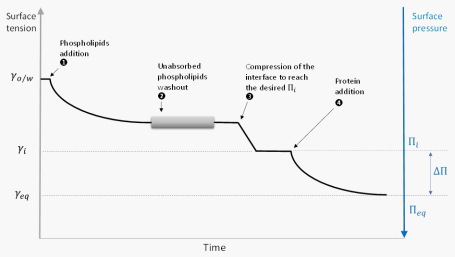

Figure 1 : overview of an experiment to determine the surface pressure increment ΔΠ for one initial pressure Πi

To determine the exclusion pressure Π_e, the experimental curve $\Delta\Pi$ as a function of Π_i is fitted linearly as shown in Figure 2. The value of Π_e corresponds to the surface pressure at which $\Delta\Pi$ is equal to 0. For $\Pi_i < \Pi_e$, the protein is able to adsorb at the interface and causes an increase in additional surface pressure $\Delta\Pi$. When $\Pi_i > \Pi_e$ the protein can no longer adsorb at the interface.

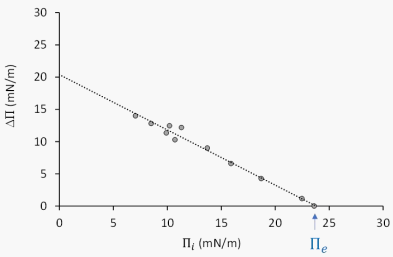

Figure 2 : surface pressure increment ΔΠ as function of the initial surface pressure Πi

The value of Π_e obtained for this protein (23.8 mN/m) illustrates its high affinity for a monolayer of phospholipids. This Π_e value is typical for penetrating proteins such as digestive lipases [7]. Other proteins, like lysozyme, have a Π_e of only 11.9 mN/m when interacting with a monolayer of egg PC which indicates their weak affinity for this type of phospholipids [8].

CONCLUSION

The oil drop tensiometer Tracker™ determines the exclusion pressure of a protein which is an indicator of its ability to penetrate an interface. Interfaces of the same chemical composition but of different surface concentrations are prepared with the Tracker™. The additional decrease of surface tension induced by the protein is an evidence of its incorporation into the interface. Above a critical value of surface pressure Π_i , corresponding to the exclusion surface pressure Π_e , this adsorption is no longer possible and no further lowering of surface tension is observed (ΔΠ=0).

Table 1 : exclusion pressures of molecules at lipid interfaces.

Type of molecule	Domains and applications	Molecule of interest	Monolayer[1]	Π_e (mN/m)	Ref.
Snake venom peptide	Research	Cardiotoxin	DLPG	45	[9]
Antimicrobial peptide	Antiviral, antibacterial and hemolytic activities	Surfactin S15	DMPC	43	[10]
Peptide of a neurodegenerative disease (Alzheimer's disease)	Research, Protein destructuring	Amyloid β-peptide	Sphingomyelin	55	[11]
Low molecular weight protein	Research	FABP (Fatty acid binding protein)	POPG	29	[12]
Blood lipoprotein protein	Research, maturation of HDL (High density lipoproteins)	Apolipoprotein C-1	POPC	34,4	[2]
Lipopeptide	Research, Health, pulmonary surfactant	N-terminal segment of the SP-C protein	DPPC	37	[13]
Antibody	Research, study of antibody-antigen complexation	IgG	DPPC/DPPA/NBD -PE	28	[14]
Muscle cell protein	Research, Health	DYS R16-21 (fragment of dystrophin)	DOPC/DOPS	26,5	[15]
Phospholipase	Research, Health (inflammatory response)	cPLA$_2\alpha$ (Human cytosolic phospholipase A$_2$)	D-POPC	21	[16]
Protein	Research, Health (activation of the immune system)	Calcineurin CaN	POPC	25	[17]
Neuropeptide	Research, Neurobiology	NPY	DMPS	36	[6]
Fungal lipase	Enzymatic detergency	SAL3 (*Staphyloccoccus aureus* lipase 3)	DLPC	37	[18, 19]
Constituent for formulation	Cosmetics, Health, Food	Lysozyme	DPPC	22,6	[20]
Emulsifier	Food	β-casein	DPPC	21,3	[21]
Milk protein	Food	Milk lipoprotein lipase	Monolayer of milk fat globule membrane	25	[22]
Digestive lipase	Food, Health	HPL (Human pancreatic lipase)	Egg PC	15	[23]

1 For the complete description of the interface model, refer to the corresponding articles.

References

1. Calvez, P., et al., Parameters modulating the maximum insertion pressure of proteins and peptides in lipid monolayers. Biochimie, 2009. 91(6): p. 718-33.
2. Meyers, N.L., et al., Changes in helical content or net charge of apolipoprotein C-I alter its affinity for lipid/water interfaces. J Lipid Res, 2013. 54(7): p. 1927-38.
3. Wang, L., D. Atkinson, and D.M. Small, The interfacial properties of ApoA-I and an amphipathic alpha-helix consensus peptide of exchangeable apolipoproteins at the triolein/water interface. J Biol Chem, 2005. 280(6): p. 4154-65.
4. Wang, L., et al., The N-terminal (1-44) and C-terminal (198-243) peptides of apolipoprotein A-I behave differently at the triolein/water interface. Biochemistry, 2007. 46(43): p. 12140-51.
5. Nylund, M., et al., Membrane curvature effects on glycolipid transfer protein activity. Langmuir, 2007. 23(23): p. 11726-33.
6. Dyck, M. and M. Losche, Interaction of the neurotransmitter, neuropeptide Y, with phospholipid membranes: film balance and fluorescence microscopy studies. J Phys Chem B, 2006. 110(44): p. 22143-51.
7. Benarouche, A., et al., New insights into the pH-dependent interfacial adsorption of dog gastric lipase using the monolayer technique. Colloids Surf B Biointerfaces, 2013. 111: p. 306-12.
8. Benarouche, A., et al., Interfacial Properties of NTAIL, an Intrinsically Disordered Protein. Biophys J, 2017. 113(12): p. 2723-2735.
9. Bougis, P., et al., A possible orientation change of cardiotoxin molecule during its interaction with phospholipid monolayer. Toxicon, 1982. 20(1): p. 187-90.
10. Eeman, M., et al., Penetration of surfactin into phospholipid monolayers: nanoscale interfacial organization. Langmuir, 2006. 22(26): p. 11337-45.
11. Mahfoud, R., et al., Identification of a common sphingolipid-binding domain in Alzheimer, prion, and HIV-1 proteins. J Biol Chem, 2002. 277(13): p. 11292-6.
12. Nolan, V., et al., Interactions of chicken liver basic fatty acid-binding protein with lipid membranes. Biochim Biophys Acta, 2003. 1611(1-2): p. 98-106.
13. Plasencia, I., K.M. Keough, and J. Perez-Gil, Interaction of the N-terminal segment of pulmonary surfactant protein SP-C with interfacial phospholipid films. Biochim Biophys Acta, 2005. 1713(2): p. 118-28.
14. Wang, H., et al., Assembly of antibodies in lipid membranes for biosensor development. Appl Biochem Biotechnol, 1995. 53(2): p. 163-81.
15. Ameziane-Le Hir, S., et al., Cholesterol favors the anchorage of human dystrophin repeats 16 to 21 in membrane at physiological surface pressure. BBA-Biomembranes, 2014. 1838(5): p. 1266-73.
16. Lichtenbergova, L., E.T. Yoon, and W. Cho, Membrane penetration of cytosolic phospholipase A2 is necessary for its interfacial catalysis and arachidonate specificity. Biochemistry, 1998. 37(40): p. 14128-36.
17. Kennedy, M.T., H. Brockman, and F. Rusnak, Contributions of myristoylation to calcineurin structure/function. J Biol Chem, 1996. 271(43): p. 26517-21.
18. Horchani, H., et al., Heterologous expression and N-terminal His-tagging processes affect the catalytic properties of staphylococcal lipases: a monolayer study. J Colloid Interface Sci, 2010. 350(2): p. 586-94.
19. Horchani, H., et al., Biochemical and molecular characterisation of a thermoactive, alkaline and detergent-stable lipase from a newly isolated Staphylococcus aureus strain. J Mol Catal B-Enzym, 2009. 56(4): p. 237-45.
20. Torrent Burgués, J., & Raju, R., Effect of lysozyme subphase and insertion on several lipid films. Adv Mater Sci, 2019. 4(1): p. 1-7.
21. Carota, F., et al., Neural dynamics of the intention to speak. Cereb Cortex, 2010. 20(8): p. 1891-7.
22. Danthine, S. and C. Blecker, Interactions of lipases with milk fat globule membrane monolayers using a Langmuir film balance. Int Dairy J, 2014. 35(1): p. 81-7.
23. Chahinian, H., et al., The beta 5' loop of the pancreatic lipase C2-like domain plays a critical role in the lipase-lipid interactions. Biochemistry, 2002. 41(46): p. 13725-35.