

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: $\Pi = \gamma_0 - \gamma$

where γ_0 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 $\gamma_{(o/w)}=32 \text{ mN/m}$. 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 γ_i . 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 $\Delta\Pi=0 \text{ mN/m}$, this means that the surface pressure Π_i is too high for the protein to adsorb at the interface. When $\Delta\Pi=0$, $\Pi_{eq}=\Pi_i$.

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.

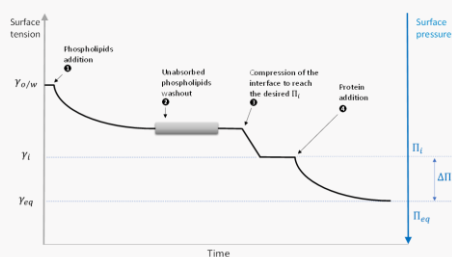


Figure 1 : overview of an experiment to determine the surface pressure increment $\Delta\Pi$ for one initial pressure Π_i

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.

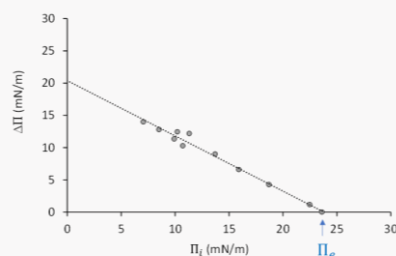


Figure 2 : surface pressure increment $\Delta\Pi$ as function of the initial surface pressure Π_i

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 ($\Delta\Pi=0$).