How to determine the maximum surface pressure of a molecule

INTRODUCTION

The drop tensiometer TRACKER™ can measure the ability of a molecule to bind an interface, to remain adsorbed and/or to be ejected there. A parameter noted Π_{MAX}, highlighted by D. Small's research group, allows to determine the maximum pressure that peptide, once established at the interface, can withstand before being ejected back into the aqueous phase [1]. Table 1 shows examples of Π_{MAX} values, calculated for different peptides. These values were obtained at oil/aqueous phase interfaces, in presence or in absence of phospholipids.

METHODOLOGY

Maximum surface pressure was determined by the sequence of compression dilatation steps. Addition of either the protein A or the protein B lowered the surface tension to a value noted γ_i and a surface pressure noted Π_i: $\Pi_i = \gamma_{o/w} - \gamma_i$ The aqueous phase was replaced by a fresh buffered solution to remove the non-adsorbed proteins. The volume of the drop was decreased by the TRACKER™ drop tensiometer to reach a desired surface pressure noted Π_0 and defined as following: $\Pi_0 = \gamma_{o/w} - \gamma_0$ where γ_0 represented the surface tension after the compression. The ejection of the protein induced a decrease of the surface pressure. This equilibrium surface pressure was defined as shown in the equation: $\Pi_{eq} = \gamma_{o/w} - \gamma_{eq}$ where γ_{eq} represented the surface tension at the equilibrium after a relaxation time. The change in surface tension noted $\Delta\gamma$ was defined as: $\Delta\gamma = \gamma_{eq} - \gamma_0$

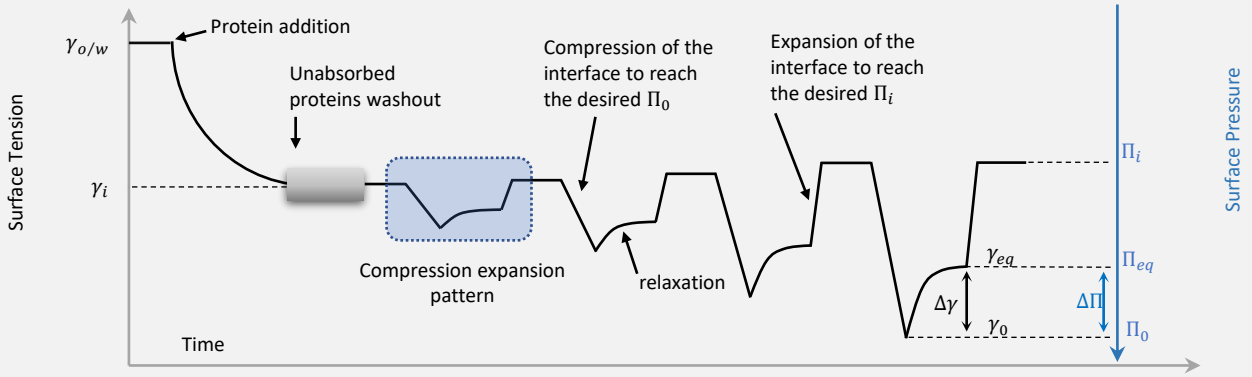

Figure 1: overview of the successive compression/expansion pattern used to determine the maximum surface pressure

EXPERIMENTAL PROTOCOL

A drop of triolein of 20 µL was 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 initial interfacial tension γ_{ow}=32 mN/m. Then, a solution containing a protein of interest was injected in the aqueous phase. Results with two proteins, protein A and protein B, were showed.

RESULTS

The range of values of $\Delta\gamma$ was an indicator of the adsorption and desorption capacity of a protein.

Indeed, a positive surface tension increment indicated that the bound protein molecules had desorbed from the surface. If there was no change in surface tension, it indicated that the protein remains at the surface. A negative surface tension increment indicated that the protein in the bulk solution was still able to adsorb onto the surface.

Proteins A and B exhibited two different behaviors at the oil/aqueous phase interface. Positive $\Delta\gamma$ values were obtained for the protein A, bringing out its desorption from the triolein/aqueous phase interface, whereas for the protein B, no change in surface tension was recorded, corresponding to a strong binding at the interface.

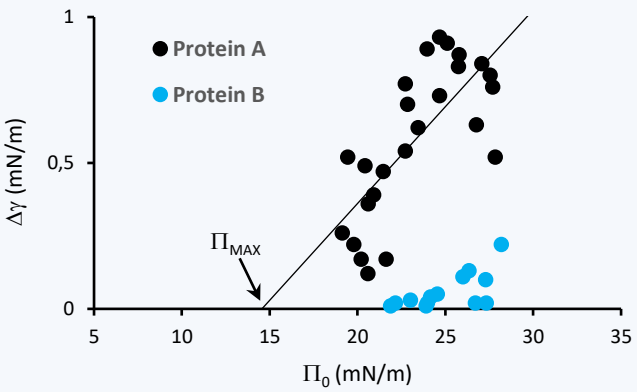

Figure 2: surface tension increment $\Delta\gamma$ as function of the initial surface pressure Π_0 for proteins A and B.

RESULTS

The maximum surface pressure (Π_{MAX}) was graphically determined by plotting $\Delta\gamma$ as the function of Π_0 (Figure 2). The linear regression intercepted at $\Delta\gamma = 0$ and gave the Π_{MAX} of the protein A, with a value of 14.6 mN/m.

Π_{MAX} was used to rank proteins. To push off the protein A from the interface, a surface pressure of 14.6 mN/m had to be applied. Unlike protein B, no Π_{MAX} value could be calculated, This result has been attributed to a very high value of Pi max ($\Delta\gamma$ closed to zero), suggesting a strong affinity for triolein/aqueous phase interface.

Π_{MAX} was also used to evaluate the affinity of a different proteins for different interfaces. Table 1 showed Π_{MAX} of different peptides and interfaces. In comparison with other peptides studied so far at the triolein/aqueous phase interface (Table 1), the Π_{MAX} value obtained for protein A was in the same range. Interestingly, integral peptides (perilipin [2], apolipoproteins [3],[4]) showed a better affinity for interfaces laden with phospholipids rather than pristine oil/water interface.

	P_{max} (mN/m)		Ref.
Peptide	*Triolein/water*	*Triolein/POPC/water*	
Human apoA-I	17.5		[5]
N-terminal (1-44) peptide of apoA-I (N44)	13.2		[6]
C-terminal (198-243) peptide of apoA-I (C46)	16.2		
C-terminal (198-243) peptide of apoA-I (C46)	BWO: 16.2/PWO: 13.2		[7]
D(185-243)apoA-I	17.4		[5]
D(1-59)(185-243)apoA-I	16.5		
Apolipoprotein B-100	13.0		[8]
ApoB6.4-177	BWO: 16.9 ±0.5/PWO: 16.5		[9]
ApoB6.4-138	16.7		[10]
ApoB13-179	19.2		
ApoB37-41	16.0		[11]
ApoC-I	16.8	20.7	[3]
ApoC-I G15A	18.0	22.6	[4]
ApoC-I G15P	16.1	20.0	
ApoC-I R23P	16.4	18.2	
ApoC-I M38P	16.0	19.5	
Plin1 11mr-containing domain (aa 93–192) WT	16.6	19.1	[2]
Plin1 11mr-containing domain (aa 93–192) mutant (L143D)	13.6	17	

BWO: Before wash-out
PWO: Post wash-out

Table 1 : Π_{max} values for different proteins and peptides.

CONCLUSION

Information on adsorption and ejection of molecules from an interface can be obtained using the drop tensiometer TRACKER™. Once the adsorption of molecules at an interface is achieved, cycles of compressions/dilations allow to calculate the change in surface tension ($\Delta\gamma$). The relaxation of the surface tension after the compression step is an evidence of its departure/reorganization at the interface. There is a surface pressure from which a tiny surface tension increment will correspond to the departure of proteins from the interface. It is the Π_{MAX}.

References:

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