Peptides

Copenhagen 2010: Tales of Peptides

The Proceedings of the Thirty-First European Peptide Symposium

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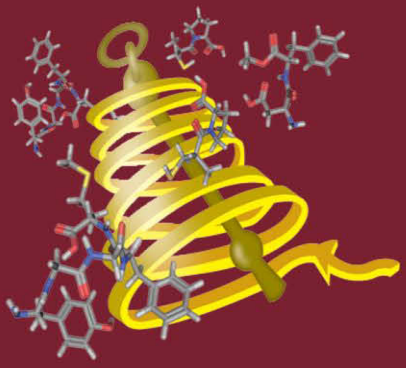

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Quantifying Molecular Partition of Charged Molecules by Zeta-Potential Measurements

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Introduction

Many cellular phenomena occur at the biomembranes level. Biomolecules, such as several peptides (e.g., antimicrobial peptides - AMP) and proteins, exert their effects at the cell membrane level. This feature creates the need of investigating their interactions with lipids to clarify their mechanisms of action and side effects [1].

The determination of molecular lipid/water partition constants (K_p) is frequently used to quantify the extension of the interaction, which has been achieved by using different spectroscopic and chromatographic methodologies [2-4].

In this work, we derived and tested a mathematical model to determine the K_p from ζ-potential data [3]. The values obtained with this method were compared with those obtained by fluorescence spectroscopy, a regular technique used to quantify the interaction of intrinsically fluorescent peptides with selected biomembrane model systems.

Two antimicrobial peptides (BP100 and pepR [5,6] were evaluated by this new method. The results obtained by this new methodology show that ζ-potential is a powerful technique to quantify peptide/lipid interactions of a wide variety of charged molecules, overcoming some of the limitations inherent to other techniques, such as the need for fluorescent labeling.

Results and Discussion

The quantification of partition can be tackled considering the distribution of a solute between immiscible phases [2]. Briefly, the partition constant can be formulated by equation 1 [2]. When using fluorescence spectroscopy, the variation of the fluorescence intensity emitted by the peptide with the concentration of lipid ([L]), is a linear combination of the contribution of the molecules in the aqueous or on the lipid phase [2]. The fraction of membrane-bound (X_L) solute can be related to K_p by Equation 2:

$$K_P = \frac{[P]_L}{[P]_W} \Leftrightarrow K_P = \frac{\frac{n_{s,L}}{V_L}}{\frac{n_{s,W}}{V_W}} \qquad \text{Eq. 1.}$$

$$\frac{I_f}{I_W} = \frac{1 + K_P \gamma_L [L] \frac{I_L}{I_W}}{1 + K_P \gamma_L [L]} \qquad \text{Eq. 2.}$$

Where K_p represents the partition constant, $n_{s,i}$ and $[P]_i$ are respectively the moles of solute and the peptide concentration in each environment i (i = W, water; i = L, Lipid) and γ_L is the molar volume of the lipid. I_W and I_L are the fluorescence intensities arising when all the peptide is in the aqueous and on the lipid phases, respectively.

Here we present a mathematical formalism to determine partition constants of polyelectrolytes, such as charged peptides (a characteristic shared by almost all AMP for instance), to lipid vesicles (LUVs) by ζ-potential measurements [3,7]. The basic assumption of this method is that the particle under study can be electrophoretically displaced, which is a consequence of its global net charge [7]. This method relies on electrostatic interactions, which have an important role in partition phenomena [3,8].

To determine the K_P value by this methodology, it is necessary to know in advance the net charge of the peptide and the proportion and effective charge of the ionic phospholipids in the vesicles. The reduction of global net charge of LU Vs induced by the peptide is proportional to the fraction of charged lipids that are neutralized, given by Equation 3:

In Equation 3 $|\Delta\zeta/\zeta_0|$ is the relative reduction in ζ-potential, which is dependent on the global peptide concentration, [P]; $n_{PL_{neutralized}}$ is the number of charged phospholipids that

Table 1. Values of the partition constants of BP100 and pepR to liposomes with different lipid compositions, at pH 7.4, obtained by ζ-potential and fluorescence spectroscopy measurements. (K_p ± standard deviation)

Peptide	*Lipid Mixture*	$K_p / 10^3$	
		Fluorescence Spectroscopy	*ζ-Potential*
BP100	POPC:POPG 1:2	50.7 ± 9.30	56.7 ± 0.70
pepR	POPC:POPG 4:1	0.44 ± 0.08	1.70 ± 0.06
	POPC:POPG 3:2	4.50 ± 1.06	5.50 ± 1.30

were perturbed by the peptide interaction and $n_{PL_{total}}$ is the total amount of charged phospholipids. Assuming that each nominal peptide charge, when interacting with the lipid membrane, neutralizes one nominal phospholipid charge, it is possible rearrange Equation 3 to Equation 4. z_{lipid} is the absolute charge of the lipid and ζ_{final} is the ζ-potential value for each titration with peptide. Plotting ζ_{final}/ζ_0 vs [P], it is possible to determine the value of K_P from the slope, using and Equation 4, where X_L is a function of K_P (*2*).

$$\left|\frac{\Delta\zeta}{\zeta_0}\right| = \frac{n_{PL^-_{neutralized}}}{n_{PL^-_{total}}} \qquad \text{Eq. 3.}$$

$$\frac{\zeta_{final}}{\zeta_0} = 1 + \frac{X_L z_{Peptide}}{f_{PL}[L] z_{Lipid}}[P] \qquad \text{Eq.4.}$$

We developed a new mathematical formalism to determine K_p from ζ-potential data and compared the retrieved values with those obtained by fluorescence spectroscopy. The K_p values calculated are summarized in Table 1. The net charges considered were -1, +12 and +6 for the anionic phospholipids, pepR and BP100 [6], respectively. Both methods retrieved comparable K_p values. ζ-potential measurements prove to be a reliable method for studying peptide/lipid interactions and for K_p determination. ζ-potential is a powerful approach when studying charged molecules that are also good scatterers; however charged particles are mandatory. When using fluorescence spectroscopy, a fluorescent reporter in the membrane-active molecule is needed. Nevertheless, there are some cases where no reporter is available in the native molecule. With this novel approach, it is possible to quantify the partition of molecules with no intrinsic fluorescent reporter, as long as they are charged, which is an almost universal condition in some areas of study, such as antimicrobial peptides.

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References
1. Reddy, K., et al. *Int. J. Antimicrob. Agents* **24**, 536 (2004).
2. Santos, N.C., et al. *Biochim Biophys Acta* **1612**, 123 (2003).
3. Domingues, M.M., et al. *J. Pep. Sci.* **14**, 394 (2008).
4. Matos, P.M., et al. *Biochim Biophys Acta* **1798**, 1999 (2010).
5. Alves, C., et al. *J. Biol. Chem.* **285**, 27536 (2010).
6. Ferre, R., et al. *Biophys. J.* **96**, 1815 (2009).
7. Kaszuba, M., et al. *Philos Transact. A Math Phys. Eng. Sci.* **368**, 4439 (2010).
8. White, S., Wimley, W. *Biochim Biophys Acta* **1376**, 339 (1998).