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Study of the miscibility of the hepatitis A synthetic antigen [Lys]^{113}VP3110 with lipids

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Abstract. The miscibility of the synthetic peptide antigen of hepatitis A, [Lys]^{113}VP3110 with zwitterionic, anionic and cationic lipids was studied through compression isotherms of monolayers at the air/water interface.
Deviations from ideality was found, with a different behaviour to the one observed with the parent peptide VP3(110-121). Deviations were quantified through the calculation of thermodynamic parameters such as the free energy of mixing (ΔG^{EX}_{M}), interaction parameter (α) and the enthalpy (ΔH).

1. INTRODUCTION

Previous studies carried out in our group have shown that the replacement of the Glycine at position 113 by Lysine in the continuous epitope of hepatitis A virus, VP3(110-121): FWRGDLVFDFDV [1], does not induce a significant loss of peptide recognition by human convalescent sera. When studied the reactivity of a panel of anti-HAV human sera from the Valdivia General Hospital of Chile, similar results of reactivity and sensitivity were obtained [2] .
In the present paper we describe and discuss the factors affecting the miscibility of [Lys^{113}]-VP3(110-121) with lipids of different net charge (Dipalmitoylphosphatidylcholine, Dipalmitoylphospatidylglycerol and Stearylamine).

2. MATERIAL AND METHODS

2.1.Materials

Dipalmitoylphosphatidylcholine (DPPC), Dipalmitoylphosphatidylglycerol (DPPG) and Steraylamine (SA), were purchased from Sigma Chemical Co. The subphase was phosphate-buffered saline pH 7.4 (PBS) (0.017 M $NaH_2PO_4 \cdot 2H_2O$, 0.081 M $Na_2HPO_4 \cdot 12H_2O$, 0.05 M NaCl). Stock peptide solutions of 1 mM were prepared in DMSO/water; the peptide was first dissolved in pure DMSO (dimethylsulphoxide, ACS reagent ≥ 99.9% Sigma Chemical Co),then diluted to the final concentration.

2.2.Methods

Measurements were performed on a Langmuir film balance, KSV5000, equipped with a Wilhelmy platinum plate. Pressure-area isotherms were performed in a Teflon trough (surface area 17000 mm^2, volume 1000 ml).
Compression isotherms of monolayers on PBS were formed monolayers from solutions of the lipid and its mixtures with the peptide on the PBS subphase using a microsyringe (Hamilton, Co). After allowing 15 minutes for stabilization, the monolayer was compressed (symmetrical compression) with an area reduction rate of 60 mm^2/min. The films were compressed up to their collapse pressure. Each run was repeated three times and the reproducibility was ± 0.01 nm^2/molecule.

3.Results and discussion

[Lys]^{113}VP3110, was able to form stable monolayers at the air/water interface. (Figure 1).

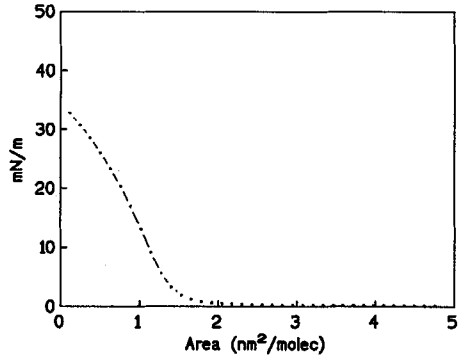


Figure 1. Compression isotherm of [Lys]^{113}VP3110, spread on PBS subphase.

Area values at the collapse (table 1) suggest that probably the peptide has a β sheet conformation [3].

Table 1. Characteristics of the [Lys]^{113}VP3110 compression isotherm.

Collapse Pressure (mN/m)	Collapse Area (nm^2/molecule)	Area at π =0 (nm^2/residue)	Area at the "lift-off" (nm^2/residue)
29	0.52	0.08	0.12

[Lys]^{113}VP3110/lipid mixed monolayers

Compression isotherms of [Lys]^{113}VP3110/lipid mixed monolayers showed in general a intermediate behaviour between the pure components. The presence of the peptide in the monolayers, even at low concentrations (0,8 molar fraction) produces an abolishment of the phase transition in DPPC and DPPG isotherms. As an example, figure 2 shows [Lys]^{113}VP3110/DPPC mixed monolayers.

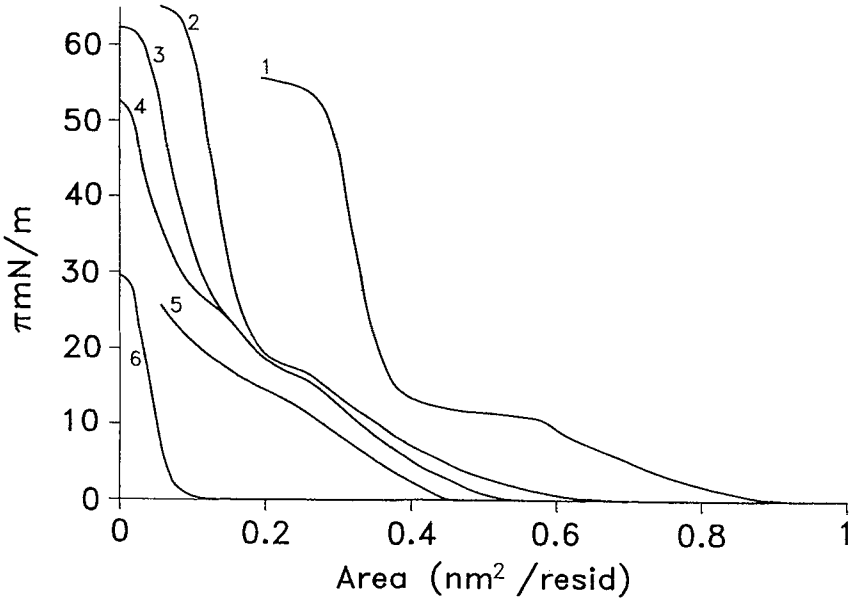
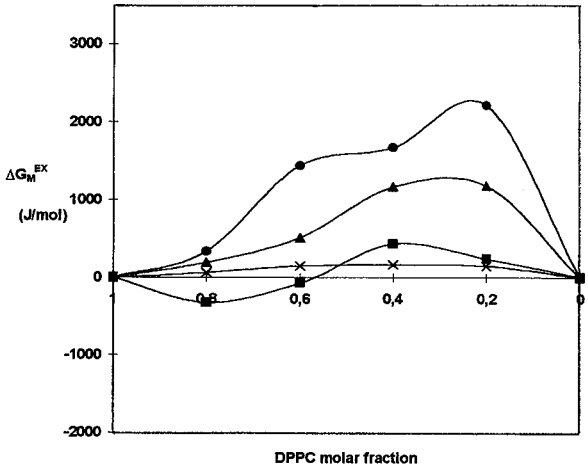
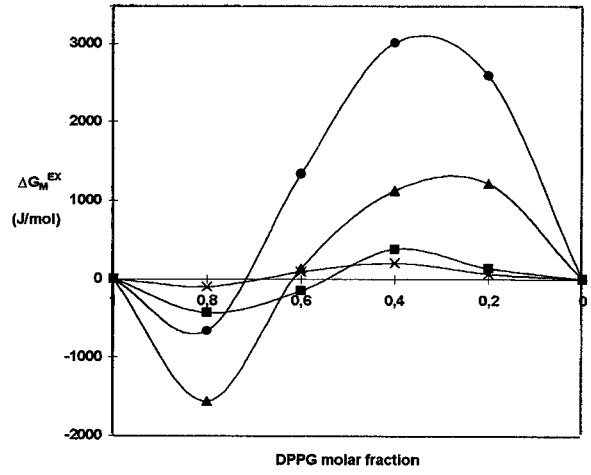


Figure 2 Compression isotherms of [Lys]^{113}VP3110/DPPC mixed monolayers.
1-DPPC, 2-DPPC 0.8/-Lys]^{113}VP3110 0.2, 3- DPPC 0.6/-Lys]^{113}VP3110 0.4,
4- DPPC 0.4/-Lys]^{113}VP3110 0.6, 5- DPPC 0.2/-Lys]^{113}VP3110 0.8, 6- [Lys]^{113}VP3110.

In the three mixed monolayers deviations from ideallity were found. For DPPC and DPPG monolayers deviations are in general positive in general but some negative deviations were found for monolayers containing low proportions of peptide. In SA mixed monolayers deviations were positive in all cases but for 0.8 peptide containing monolayer at 30 mN/m. All these deviations were quantified thorough the calculation of free energy of mixing (ΔG^{EX}_M), interaction parameter (α) and enthalpy (ΔH) as described in [4]. As expected these values were specially high for DPPG and SA monolayers at 20 and 30 mN/m (Figure 3).
To have a closer understanding of these deviations, miscibility calculations using Crisp rule [4] was undertaken for collapse and change phase pressures. Values obtained were consistent with the existence of miscibility between the peptide and the three lipids assayed. This behaviour is quite different from the parent peptide VP3(110-121) which showed lower values of interaction with these lipids [5]. All this suggest that the

replacement of the Glycine at position 113 by Lysine, produce some changes in th peptide conformation that influences its miscibility with lipids.





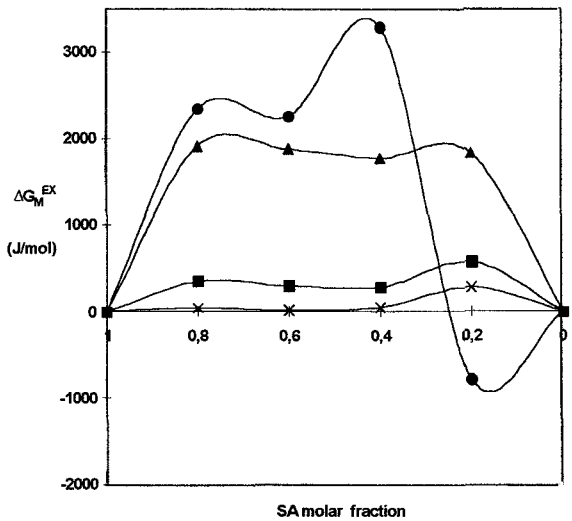


Figure 3. Representation of ΔG^{EX}_M values in front of molar fraction for the three mixtures assayed. ● 5 mN/m, ▲ 10 mN/m, ■ 20 mN/m, ×30 mN/m.

4.References

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