Interaction of a hepatitis A synthetic antigen 
$[^{105,109}\text{ABU}]\text{VP3(101-121)}$ with lipid monolayers

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Abstract. We describe the synthesis and characterization of an antigenic peptide: $[^{105,109}\text{ABU}]\text{VP3(101-121)}$, corresponding to the (101-121) sequence of VP3 protein from Hepatitis A virus. The physicochemical properties of the peptide were studied using lipid monolayers as a model membrane by means the Langmuir-Blodgett film technique. The main goal of the research was to get insight into the possible mechanism of action of the peptide at the membrane level and therefore, to better understand the proliferation and infection processes of the virus in the host. For that reason, interaction of the peptide with neutral, anionic and cationic phospholipids was studied by the determination of the penetration kinetics and compression isotherms. The peptide had surface activity, concentration dependent and was able to incorporate into lipid monolayers.

1. INTRODUCTION

Synthetic peptides have shown to be of great interest in the generation of immuno response specially when incorporated into liposomes. For that reason, peptide synthesis has been developed and improved by following different strategies [1].

On another hand, model membranes like lipid monolayers have been extensively used to analyze the interaction of bioactive molecules with lipids [2]. They are a useful tool to get insight into the affinity of a molecule versus a lipid. In that sense, several thermodynamic parameters as excess free energy of mixing ($\Delta G_m^{\text{ex}}$), interaction parameters ($\alpha$) or enthalpy ($\Delta H$) can be obtained by using lipid monolayers spread at the air/water interface [3].

Taking into consideration these findings, we have synthesized and characterized the $[^{105,109}\text{ABU}]\text{VP3(101-121)}$ peptide corresponding to the highly immunogenic VP3 (110-121) sequence of the Hepatitis A virus. In a second step, we have used the Langmuir-Blodgett technique to study first the ability of the peptide to form monolayers at the air/water interface and then its interaction with dipalmitoylphosphatidylcholine (DPPC).

2. EXPERIMENTAL PART

2.1. Monolayer studies

Measurements were recorded using a Langmuir film balance KSV5000, equipped with a Wilhelmy platinum plate as described by Verger [4].

2.2. Surface activity measurements were carried out using a cylindrical Teflon trough of 70 ml capacity with mechanical stirring. Increasing volumes of a concentrated solution of the peptide were injected beneath the surface through a lateral whole. The increase of surface pressure ($\Delta \pi$) with time was recorded until a steady-state value of $\Delta \pi$ was obtained.
2.3. Compression isotherms

To carry out these studies the output of the pressure pick-up (Sartorius micro-balance) was calibrated by recording the well-know isotherm of stearic acid. This isotherm is characterized by a sharp phase transition at 25 mN/m for a subphase of pure water at 20°C. The Teflon trough for measuring compression isotherms was rectangular in shape with a surface area 495 cm² and a volume of 330 ml. Films were spread using a microsyringe on a PBS subphase alone or with the peptide (0.315 μM) and at least 10 min were allowed for solvent evaporation. Films were compressed at a rate of 4.2 cm/min. Changes in the compression rate did not alter the shape of the isotherms. Standard deviation for these measurements was ≤ 3.5%.

3. RESULTS AND DISCUSSION

3.1 Peptide synthesis

The synthesis of protected HAV-[105,109 Abu]VP3(101-121) was accomplished by using an ortogonal strategy based on the attachment of the Riniker handle (HMPB) to MBHA resin as previously described (1). This method allows the specific cleavage of the peptide sequence from the polymeric support without side chain deprotection.

Semi preparative HPLC was used for purification of crude peptides. The purity achieved was higher than 95%. As shown in Table 1 the peptide was characterised by analytical HPLC, amino acid analysis and electrospray mass spectrometry (Fig 1).

Table 1. Analytical data of HAV-[105,109 Abu]VP3(101-121)

<table>
<thead>
<tr>
<th>Amino acid analysis*</th>
<th>HPLC (tR)**</th>
<th>ES-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>D=2.1 (2); S=0.6 (1)</td>
<td>20 min</td>
<td>[M]⁺ = 3124.6</td>
</tr>
<tr>
<td>Q=2.1 (2); G=1.0 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A=1.0 (1); V=2.1 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M=0.8 (1); L= 0.9 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L= 1.9 (2); F=3.7 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R= 1.0 (1); W n.d.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Theoric values in parenthesis

** HPLC conditions: Spherisorb ODS column (10 μm) eluted with acetonitrile (ACN)/water (0.05% TFA) mixtures. 1) 5 min isocratic of 70% ACN. 2) Gradient from 70% ACN to 100% ACN in 15 min. 3) Final isocratic step: 100% ACN 10 min.

Figure 1. Electrospray mass spectrometry of HAV-[105,109 Abu]VP3(101-121). [M⁺]=3124.3
Due to the fact that for the majority of identified proteins the only structural information available is the amino acid sequence, empirical rules for predicting the position of continuous epitopes in proteins from several features of the primary structure have been developed. For predicting the position of continuous epitopes, the highest peaks appearing in hydrophilicity plots should be considered. In that sense, Figure 2 shows the hydrophilicity profile of the peptide calculated according to the Hopp and Woods method. Results are indicative of a predominance of hydrophilicity.

![Graph showing hydrophilicity profile.](image)

**Figure 2.** Hydrophilicity profile of HAV-[\textsuperscript{105,109}Abu]VP3(101-121).

Moreover it has been shown that methods for predicting the secondary structure of proteins can be used as predictors of antigenicity. Algorithms that predict the position of loops or turns at the same time predict regions of highest hydrophilicity. In our case, we have used the Chou and Fasman [5] semiempirical method. As can be seen in Fig.3, there is a predominance of $\beta$-structures ($\beta$-turn and $\beta$-sheet).

![Graph showing conformational profile.](image)

**Figure 3.** Conformational profile of HAV-[\textsuperscript{105,109}Abu]VP3(101-121). --- helix, --- sheet --- turn.
3.2. Surface activity

Experiments were performed as described by Mota et al [3]. Increasing amounts of peptide were injected into the PBS subphase (PBS, pH 8.4) until saturation and the time course of pressure increases was registered. Peptide adsorption in the interface air/water was concentration dependent until the saturation point or concentration that gave the maximum surface pressure increase. There is a tendency towards saturation at high concentrations, thus suggesting the existence of an equilibrium between the concentration of drug molecules in solution and the fraction of them in the surface. Results are shown in Fig 4.

![Surface activity graph](image)

**Figure 4.** Surface activity of increasing amounts of HAV-[105,109]Abu] VP3(101-121) injected into the PBS (pH 8.4) subphase.

3.3 Compression isotherms

The interaction of the peptide with DPPC was checked by spreading monolayers composed of different molar fractions of peptide/DPPC on a PBS subphase. Initially, monolayers of the peptide were prepared either by injecting a solution of this peptide into the aqueous subphase or spreading the peptide on the surface. After compression, both isotherms were almost identical which indicates that the method of injection did not affect the surface characteristics of the monolayer.

Isotherms were obtained for all the mixtures after monolayer compression. Collapse was reached only for DPPC isotherms. Results are shown in Figure 5. A clear expanding effect with no clear transition form liquid-condensed to expanded phases is observed for the lipid to peptide ratio of 0.8 and 0.6, maybe due to the formation of different lipid-peptide domains.
Figure 5. Compression isotherms of HAV-[\textsuperscript{105,109}Abu]VP3(101-121)/DPPC spread on PBS subphase.

References