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# Surface behaviour and peptide–lipid interactions of the E1(3-17)R and E1(3-17)G peptides from E1 capside protein of GBV-C/HGV virus

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ABSTRACT

The Hepatitis G virus (GBV-C/HGV) is a parenterally transmitted virus that is frequently associated with the Hepatitis C virus (HCV) infection. In people co-infected with GBV-C/HGV and human immunodeficiency virus (HIV), a delayed progression of acquired immunodeficiency syndrome (AIDS) has been observed. The liver disease caused by GBV-C/HGV and the mechanism by which this virus may inhibit the progression of AIDS remain to be elucidated. Given that GBV-HGV is an enveloped RNA virus similar to HIV and Hepatitis B viruses, we hypothesize that, as in those viruses, capside assembly is crucial for viral infection. Thus, the study of peptides from capside proteins may help us to understand the mechanism of viral infection which takes place. Here we studied the surface behaviour of E1(3-17)R and E1(3-17)G peptides from HGV by means of the Langmuir-monolayer technique, in order to examine the peptide–membrane interaction. Both peptides showed spontaneous adsorption at the air–water interface but adsorption process was faster for E1(3-17)G peptide. In both cases, the maximum pressure reached ($\pi_{max}$) and molecular areas at maximal packing, calculated from compression isotherms of pure peptide monolayers, were in good agreement with previous reports for peptides with α-helical structures at the interface. E1(3-17)R and E1(3-17)G interacted with L-α-phosphatidylcholine (PC) and L-α-phophatidylserine (PS). Miscibility studies indicated that the two peptides interact with PC and PS; the presence of a peptide altered the shape of the compression isotherms. BAM images revealed that E1(3-17)G is immiscible with the lipids assayed. In contrast, E1(3-17)R disrupted the PS lipid film.

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## 1. Introduction

The Hepatitis G virus (GBV-C/HGV) is a parenterally transmitted virus that is frequently associated with Hepatitis C virus (HCV) infection. In people co-infected with GBV-C/HGV and human immunodeficiency virus (HIV), delayed progression of acquired immunodeficiency syndrome (AIDS) has been observed [1,2]. The liver disease caused by GBV-C/HGV and the mechanism by which this virus may inhibit the progression of AIDS remain to be elucidated. Given that GBV-HGV is an enveloped RNA virus similar to HIV and Hepatitis B virus, we hypothesize that, like those viruses, capside assembly is crucial for viral infection [3]. Thus, the study of peptides from capside proteins may help us to understand the mechanism of viral infection which takes place. We are currently examining the capacity of GBV-C/HGV synthetic peptides to interact and induce fusion in model membranes. Here we studied the surface properties of E1(3-17)R and E1(3-17)G synthetic peptides from the exposed E1 structural protein of GBV-C/HGV at the air–water interface by using the Langmuir-monolayer technique. Furthermore, we report information to explain the mechanism by which these peptides interact with membrane lipids in the context of their possible role in infection.

## 2. Materials and methods

### 2.1. Materials

E1(3-17)R ($^{2}$APATHAC*R*ANGQYFL$^{18}$) and E1(3-17)G ($^{2}$APATHAC*G*ANGQYFL$^{18}$) peptides were synthesised by solid-phase methodology at the Department of Peptide and Protein Chemistry (CSISC, Barcelona). The purity of the peptides obtained by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) was greater than 99%. The phospholipids used as membrane models were L-α-phosphatidylcholine (PC) and L-α-phosphatidyl-L-serine from bovine brain (PS), both of which were purchased from Avanti Polar lipids (Alabaster, AL). Chloroform (HPLC grade) was used as the spreading solvent for all lipids. Water was distilled twice and deionised (Milli-Q system, Millipore Corp.). The subphase was phosphate-buffered saline at

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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).

### 2.2. Monolayer studies

All monolayer experiments were performed at room temperature. Adsorption and penetration experiments were done by injecting peptides from their aqueous solution (1 mg $ml^{-1}$) into 70 ml of subphase in a 32-$cm^2$ trough. Compression isotherms were performed on a Nima (UK) Langmuir trough, equipped with a Wilhelmy plate made of filter paper. The trough (surface area: 560 $cm^2$, volume: 250 $cm^3$) and the plate were thoroughly cleaned with chloroform before each run to prevent lipid carryover. By mixing appropriate volumes of chloroform stock solutions of phospholipids (1 mg $ml^{-1}$) and of peptides (1 mg $ml^{-1}$), the lipid–peptide spreading solutions were obtained. Monolayers were formed by applying small drops of the spreading solutions on the PBS subphase (pH 7.4) with a microsyringe (Hamilton Co., Reno, NV). After 10 min, monolayers of the desired composition were continuously compressed with an area reduction rate of 20 $cm^2$ $min^{-1}$. The films were compressed to their collapse pressure when possible. Each run was repeated three times and the reproducibility was ±1 Å$^2$ molecule$^{-1}$.

### 2.3. Peptide synthesis

Peptides were synthesised manually following procedures previously described [4,5]. The syntheses were carried out on a Rink amide MBHA resin (0.64 mequiv. $g^{-1}$) by a solid-phase methodology following a Fmoc/tBu strategy by means of *N*,*N*′-diisopropylcarbodiimide (DIPCD)/1-hydroxybenzotriazole (HOBt) activation. For difficult couplings 2-(1*H*-benzotriazole-1-il)-1-3-3-tetramethyluroniumtetrafluoroborate (TBTU) and *N*,*N*-diisopropylethylamine (DEIA) agents were used. Side-protection was achieved by means of a three-fold molar excess of Fmoc-amino acids throughout the syntheses. The stepwise addition of each residue was assessed by the Kaiser's (ninhydrin) test [6]. Repeated couplings were performed when a positive ninhydrin test was obtained. A range of conditions were tested to cleave the peptide from the resin and to remove the side-chain protecting groups. Finally, peptide resins were treated with TFA (94%) solutions containing appropriate scavengers (2.5% $H_2O$, 2.5% ethanedithiol, 1% trisisopropylsilane).

Crude peptides were purified by preparative HPLC on a Shimadzu chromatograph equipped with a C8-silica column. The samples were eluted with a linear gradient of 60% $H_2O$ (0.05% TFA)/40% acetonitrile (0.05% TFA) to 40% $H_2O$ (0.05% TFA)/60% acetonitrile (0.05% TFA) in 30 min at a flow rate of 5 ml $min^{-1}$ and detected at 220 nm. Purified peptides were characterised by analytical HPLC, amino acid analysis and electrospray mass spectrometry.

### 2.4. Brewster angle microscopy

Brewster angle microscopy images were obtained on a MiniBam instrument (NFT, Göttigen, Germany) mounted on a Nima Langmuir balance. The instrument was equipped with a 30-mW laser emitting p-polarised light at 660 nm, which was reflected off the air–water interface at 53.1° (Brewster angle). The shutter speed used was 1/50s. All experiments were carried out at room temperature (21 ± 1 °C).

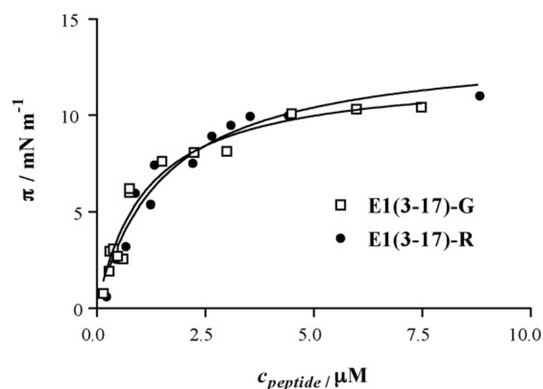


**Fig. 1.** Surface activity profile of E1 peptides. Subphase: PBS, pH 7.4.

**Table 2**
Characteristic physicochemical parameters found for E1 peptides: the experimental $\pi_{max}$, calculated $\pi_{max}$ and the constant $K$ obtained from Langmuir equation

| Langmuir equation | Peptide | $\pi_{max}$ (experimental) (mN $m^{-1}$) | $\pi_{max}$ (calculated) (mN $m^{-1}$) | $K$ (μM) |
|---|---|---|---|---|
| $\Delta\pi = \frac{c\pi_{max}}{K+c}$ | E1(3-17)R | 11.5 | 13.2 | 1.44 |
| | E1(3-17)G | 10.4 | 12.2 | 1.13 |

**Table 3**
Excess free energy and area per molecule values obtained for E1 peptides

| Peptide | $\Gamma$ (×10$^{-24}$ mol nm$^{-2}$) | $A$ (nm$^2$ molecule$^{-1}$) |
|---|---|---|
| E1(3-17)R | 1.15 | 1.44 |
| E1(3-17)G | 1.06 | 1.56 |

## 3. Results and discussion

### 3.1. Peptide synthesis

Table 1 shows the characteristic analytical parameters obtained for E1(3-17) synthetic peptides.

### 3.2. Peptide adsorption

When injected into the subphase, both E1(3-17)R and E1(3-17)G decreased the surface tension, and induced similar behaviour at the air–water interface (Fig. 1). Characteristic parameters were calculated following the Langmuir equation model [$Y = (B_{max}X)/(K_d + X)$] (Table 2). The saturating pressure values suggest that the peptides develop hydrophobic interactions when engaged at the air–water interface. The extra positive charge of the arginine residue of

**Table 1**
Analytical data of E1 synthetic peptides

| Peptide | Amino acid analysis[a] | HPLC characterization[b] | ES-MS[c] |
|---|---|---|---|
| E1(3-17)R | Y = 0.6(1); Q = 1.0(1); P = 1.0(1); G = 1.0(1); A = 4.2(4); L = 0.9(1); T = 0.9(1); F = 0.9(1); H = 0.7(1); R = 0.9(1) | $k'$ = 3.37 | $(M+H)^+$ = 1619 |
| E1(3-17)G | Y = 0.6(1); Q = 1.1(1); P = 1.3(1); G = 2.1(2); A = 4.0(4); L = 01.0(1); T = 0.9(1); F = 0.9(1); H = 0.8(1) | $k'$ = 3.68 | $(M+H)^+$ = 1518 |

[a] Theoretic values in parenthesis.

[b] Capacity factor. For both peptides the stationary phase was C8-silica column and the elution conditions were: linear gradient of 60% $H_2O$ (0.05% TFA)/40% acetonitrile (0.05% TFA) to 40% $H_2O$ (0.05% TFA)/60% acetonitrile (0.05% TFA). Flow rate of 5 ml $min^{-1}$.

[c] ES-MS electrospray mass spectrometry.

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