Biochimica et Biophysica Acta, 944 (1988) 516–520
Elsevier

BBA 70579

BBA Report

Interaction between a growth-hormone releasing hexapeptide and phospholipids spread as monolayers at the air/water interface

B. Issaurat [a,*] and J. Teissié [b]

[a] *SANOFI, Research Center in Toulouse, Toulouse*
and [b] *Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS, Toulouse (France)*

(Received 12 January 1988)
(Revised manuscript received 30 June 1988)

Key words: Growth-hormone releasing hexapeptide; Phospholipid; Monolayer; Surface fluorescence; Hydrophobic interaction; Electrostatic interaction

The interaction between a growth-hormone releasing hexapeptide and phospholipids was studied on mixed monolayers models by means of surface fluorescence. When in a monolayer this hexapeptide which contains two tryptophan molecules was observed to fluoresce. Isothermal compression experiments showed that the complex was destroyed upon compression in the case of phosphatidylethanolamine. With phosphatidylglycerol it was observed to be stable but a dramatic reversible decrease in emission was observed at high surface pressure. This is indicative of a reversible change in the organization of the peptide-phospholipid complex. These observations indicate that, in the complex, hydrophobic interactions were weak but electrostatic ones, when present, were strong enough to maintain the GHRP attached to the monolayer and not to destabilize it. The integrity of the lipid monolayer appeared not to be affected by the peptide.

The growth-hormone releasing peptide (GHRP) is a synthetic hexapeptide which stimulates in vivo and in vitro growth-hormone release [1]. It is the enkephalin analogue derived from conformational calculations which is shown to be the most active in vitro [2]. The biological interest of GHRP resides in the mechanism of growth-hormone release it allows beside the GRF-physiological releasing factor [3], its own target being not yet known.

GHRP (His-DTrp-Ala-Trp-DPhe-Lys-NH_2) is an oligopeptide composed of a non polar core and a basic amino acid at each extremity. Due to the GHRP amphiphilic structure, membrane lipids appeared as potential targets for its action. In order to check this hypothesis, we studied the behaviour of a mixed peptide lipid monolayer at the air/water interface. This membrane model is a powerful tool with which we can control the nature of chemical compounds and the physicochemical parameters. GHRP interaction with the monolayer can be monitored by an alteration of the compression isotherm and by the fluorescence of its tryptophan molecules. Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) which are known to be abundant in natural membranes were selected as host phospholipids; the PG headgroup is negatively charged but the PE headgroup is zwitterionic. Practically we compressed, in an isothermal way, peptide-lipid mixed monolayers and simultaneously measured the surface pressure and the fluorescence signal.

* Present address: 20, rue Paul Valéry, 31700 Blagnac, France.

Correspondence: J. Teissié, Centre de Recherche de Biochimie et de Génétique Cellulaires du CNRS, 118 route de Narbonne, 31062 Toulouse, France.

Egg phosphatidylcholine derived phosphatidylgycerol and *Escherichia coli* phosphatidylethanolamine were purchased from Sigma (U.S.A.). Salts were of analytical grade. Ultra pure water was obtained by a filtration device (Milli Q, Millipore France). Solvents were spectroscopically pure. The growth-hormone releasing peptide was provided by Sanofi-recherches (France). Monolayer and fluorescence experiments were performed with an interface fluorimeter [4]. The front face fluorescence of a small illuminated area (4 mm in diameter) was monitored during the compression of the film. The film surface pressure was measured by means of a platinium plate. The two parameters (fluorescence, surface pressure) were simultaneously recorded.

Experiments were carried out as follows. The signals from the subphase were first recorded and set as zero fluorescence and surface pressure. Then a mixture of the lipid and of the GHRP (at various ratios) was spread in the form of an organic solvent solution ($CHCl_3$/MeOH, 5 : 1 v/v for PE, hexane/EtOH, 9 : 1 v/v for PG). After a 3 min lag-period (which was needed for good solvent evaporation), the optical shutter was opened and the film compression was started, driven by an electric motor (0.2 nm^2/mol per min). When repeated compressions were performed, the decompression step was operated manually and a relaxation period was then observed.

In all experiments a fluorescence signal varying with compression was observed. Due to the geometry of the set-up the fluorescence was detected from the interface associated with the lipidic monolayers. The true composition of the film was not known and due to its amphiphilic character one might suggest that a fraction of the hexapeptides would leak in the subphase. As a consequence, all further experiments would refer to the composition of the spread solution.

In the case of PE-GHRP monolayers and during the first compression, for a 0.65 GHRP to PE molar ratio of the spread solution, the compression isotherm curve was almost identical to that of pure lipid. The 'observed' collapse pressure was normal, but a small residual pressure was observed at the end of the decompression (Fig. 1). The fluorescence signal increased simultaneously with the surface pressure, reached a maximum

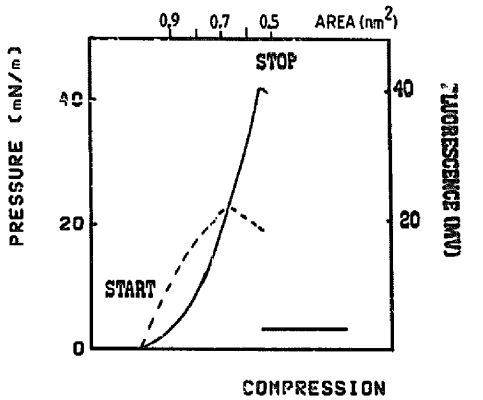

Fig. 1. Surface pressure and fluorescence changes upon compression of a PE-GHRP monolayer. The GHRP to PE molar ratio was 0.65. The subphase was pure water. Compression (from 'start' to 'stop') was monitored by the position of the moving teflon barrier (it cannot be expressed as a molecular area because the exact composition of the film is not under control as described in text). The pressure is indicated by the continuous line, the fluorescence by the dotted one. The continuous line on the right of the 'stop' is the residual pressure observed after decompression of the film. Areas on the top of the graph refer to the peptide free monolayer.

before the collapse and did not return to its initial value at the end of the compression (Fig. 1). The 'observed' collapse pressure of the monolayer decreased when the spread GHRP in PE concentration was increased beyond the 0.65 molar ratio (Fig. 3). With these high GHRP titers the compression isotherm was strongly affected and a large residual pressure was observed after decompression (data not shown). The maximum fluorescence signal increased with the titer in GHRP of the spread solution (Fig. 4), but was observed at a higher compression state; the half saturating molar ratio was about 0.32. Repetitions of compressions did not modify the compression isotherm when the spread molar ratio was smaller than 0.65 but, for higher relative concentrations in hexapeptide, such cycles induced a decrease of the 'observed' collapse pressure. At any GHRP concentration repeated compressions resulted in a sharp decrease of the fluorescence signal maximum which was divided by about 2 at each compression and took place at high apparent pressure (data now shown).

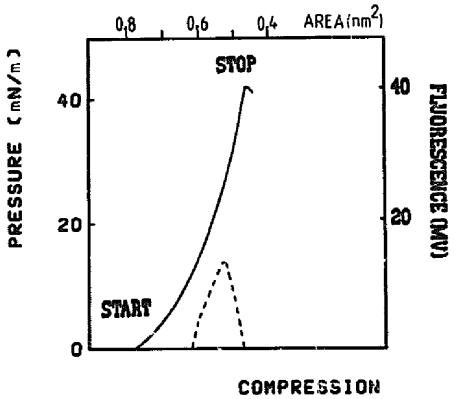

Fig. 2. Surface pressure and fluorescence changes upon compression of a PG-GHRP monolayer. The GHRP to PG molar ratio was 0.83. The subphase was 1 mM NaCl (to remove the effects of ionization on PG). Other conditions are as in Fig. 1. No residual pressure was observed after decompression. Areas on the top of the graph refer to the peptide free monolayer.

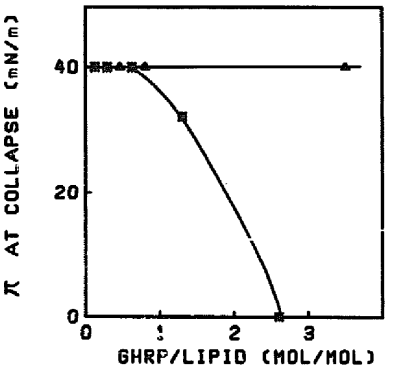

Fig. 3. 'Observed' collapse surface pressure versus the GHRP to spread lipid molar ratio. The results were obtained from the compression of different monolayers as described in Fig. 1 and 2. *, PE; ▲, PG.

In the case of PG-GHRP monolayers during the first compression, for a 0.83 spread GHRP to PG molar ratio, the compression isotherm of the mixed PG – GHRP monolayer was very similar to that of pure lipid (Fig. 2). The variation of the fluorescence occured only when the surface pressure of the monolayer was larger than 10 mN/m, reached its maximum at 25–30 mN/m and finally decreased back to its initial value before the monolayer collapse (Fig. 2). When the GHRP concentration in the spread solution was increased, the collapse pressure of the monolayer was not affected (Fig. 3) while the fluorescence signal maximum increased and reached a plateau value beyond a 0.5 molar ratio (Fig. 4), the half saturating molar ratio being about 0.16. The repetitions of the compression modified neither the compression isotherms nor the fluorescence signal behaviour. The intensity of emitted fluorescence was observed to be higher when, for the same spread GHRP to PG molar ratio, the film was compressed on a subphase at pH 6 than at pH 7.2.

The surface pressure and fluorescence results of this study are both indicative of the formation of an oligopeptide-lipid complex when the mixture is spread at the air/water interface. But the use of the platinium plate technique does not allow a quantitative determination of the interactions in the case of the neutral PE (positive charges being associated to the complex). It is well known that the platinium plate method is valid only with neutral or negatively charged films [5]. The fluorescence assay provides a reliable tool for observation of the complex. Compression of the film induces an increase in the density of molecules if no structural change was present and an increase in the emitted fluorescence should be observed. This is indeed the case in the initial steps of the compression. But with the two lipids, a dramatic

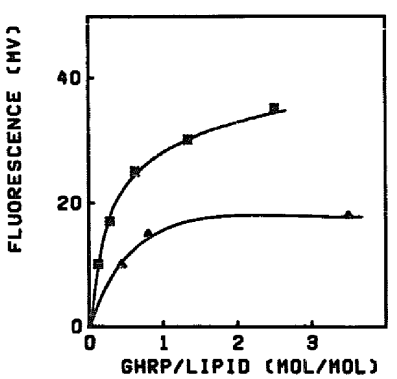

Fig. 4. Maximum surface fluorescence changes as a function of the spread GHRP to lipid molar ratio. The results were obtained from the compression of different monolayers as described in Figs. 1 and 2. *, PE; ▲, PG.

decrease is observed when the film becomes highly compressed. This is indicative of a dramatic change in the structure of the film. This drastic alteration is irreversible in the case of PE where the fluorescence changes disappear along successive decompression-compression cycles. This observation is indicative that no contribution of adsorbed or dissolved peptide to the fluorescence signal was present. It is fully reversible with PG. Previous studies using fluorescence to assay the interaction between proteins and lipids have already shown that the complex can be altered upon compression. The conformation of E 800–850 was changed when its host dimyristoylphosphatidylcholine monolayer was compressed [6] and a tilt of cardiotoxin was observed when its complex with PG reached a surface pressure larger than 25 mN/m [7]. These explanations are not valid for PE where the fluorescence changes decrease along a cycle of compression-decompression. We propose that for a given packing, the peptide is ejected from the lipid matrix and diluted away in the aqueous subphase. In the case of PG, such an ejection is not present because the dramatic change upon compression is reversible. A flipping of the peptide is likely as it was observed in the case of cardiotoxin PG complex [7]. The difference in the behaviour of the complex as a function of the nature of the host lipid can be explained when one observes the structure of GHRP. A GHRP molecule consists of a hydrophobic core with basic amino acids at both ends. Due to the zwitterionic nature of PE polar groups, the complex between PE and GHRP is stabilized mainly by hydrophobic interactions. Upon compression, these interactions are ruptured when the energy needed for the insertion of GHRP in the matrix becomes larger than its hydration energy [8,9]. As a consequence, the hexapeptide is expelled in the aqueous phase which then acts as a sink. Such an irreversible change is not observed in the case of PG which has a negatively charged polar headgroup. We can postulate that electrostatic interactions between the terminal amino acids of PG stabilize the complex along decompression-compression cycles. An indirect evidence of these electrostatic interactions is the modulation of the emitted fluorescence by the pH of the subphase. This can be due to a change in the ionization of the histidine but nevertheless one should not forget that the organization of a PG layer is under the control of the ionic content and pH of the subphase.

A molecular description of the hexapeptide-lipid complex behaviour can be obtained from the present study. According to its most bioactive conformational state [2], GHRP may be inserted in a rectangular box where the positive charges are located on one side. In the complex this side would be facing the aqueous subphase, the hydrophobic core being embedded in the hydrocarbon chains. In the case of PG (where the platinium plate is reliable), as the compression isotherms are not strongly affected, we can propose that the GHRP is located at the polar head level, such a configuration being in agreement with the electrostatic interactions between the basic amino acids and the polar groups. Upon compression, in the case of PE, GHRP leaves the hydrophobic environment at a critical surface pressure giving a strong irreversible decrease in the fluorescence emission due to a change in the orientation and in the environment of the tryptophan groups. In the case of PG, the same decrease occurs but in a reversible way, GHRP remaining bound to the polar heads, such a conformational change suggests that one of the interactions between the polar groups and the amino acids has been destroyed. Taking into account the relative polarity of lysine and histidine, it is very tempting to propose that at high surface pressure, GHRP is located in the aqueous phase remaining bound to PG by lysine amino acids. In our model one GHRP molecule interacts with two PG molecules. This is sustained by the observation of a plateau value for the fluorescence maximum just beyond a 0.5 spread GHRP in PC molar ratio (Fig. 4).

The biological implications of this study are as follows. In the case of a zwitterionic lipid, such as PE, the complex is linked to weak hydrophobic interactions and appears very difficult to form when GHRP is in aqueous phase. In the case of an acidic lipid, such as PG, the complex can be formed by electrostatic interactions and, as such, may be of a biological relevance. A very interesting feature in this case is a regulation of its configuration by the molecular packing of the host matrix. With loose lipid packing the hexapeptide is stuck against the PG monolayers but, with a

tighter packing, it is in a more erected configuration. Such a regulation of a peptide/protein–lipid monolayer interaction was previously described in the case of cardiotoxin [7] and LHCP [6]. Interestingly, in each case, a flipping of molecule seems to take place at about 25 mN/m, value which may have a biological significance.

References

1 Bowers, C.Y., Momany, F.A., Reynold, G.A. and Hong, A. (1984) Endocrinology 114, 1537–1545.

2 Momany, F.A., Bowers, C.Y., Reynold, G.A., Hong, A. and Newlander, K. (1984) Endocrinology 114, 1531–1536.

3 Guillemin, R., Braeau, P., Bohlen, P., Esch, F., Ling, N., Wehrenberg, W., Bloch, B., Mougin, C., Zeytin, F. and Baird, A. (1984) Recent Progress in Hormone Research 40, 233–299.

4 Teissié, J. (1979) J. Colloid Interface Sci. 70, 90–96.

5 Gaines, G.L. (1966) in Insoluble monolayers at liquid-gas interfaces, Wiley, New York.

6 Heck, M.W., Losche, M., Scheer, H. and Mohwald, H. (1985) Biochim. Biophys. Acta 810, 73–83.

7 Bougis, P.E., Teissié, J., Rochat, H., Piéroni, G. and Verger, R. (1987) Biochem. Biophys. Res. Commun. 143, 506–511.

8 Tandford, C. (1973) in The Hydrophobic Effect", Chap. 4, Wiley, New York.

9 Jaehnig, F. and Bramhall, J. (1982) Biochim. Biophys. Acta 690. 310–313.