

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/51377538>

Biophysical properties of a synthetic transit peptide from wheat chloroplast ribulose 1,5-bisphosphate carboxylase

ARTICLE in JOURNAL OF PEPTIDE SCIENCE · APRIL 2007

Impact Factor: 1.55 · DOI: 10.1002/psc.838 · Source: PubMed

CITATIONS

6

READS

20

3 AUTHORS:



[Ernesto E Ambroggio](#)

National University of Cordoba, Argentina

12 PUBLICATIONS 536 CITATIONS

[SEE PROFILE](#)



[Brian Maxwell Austen](#)

St George's, University of London

184 PUBLICATIONS 2,753 CITATIONS

[SEE PROFILE](#)



[Gerardo D Fidelio](#)

National University of Cordoba, Argentina

71 PUBLICATIONS 2,063 CITATIONS

[SEE PROFILE](#)

and the two sections may be functionally responsive to light or different locations within the chloroplast [3,5,6]. They contain limited regions of hydrophobic residues, many hydroxyl residues (three amino acid serine), fewer basic residues and no acidic residues [7]. Although structural-targeting peptides do not have a corrective function, they can affect the distribution of uncharged N-terminal domain of distinct regions: ~10 residues beginning with Met-Ala and terminating with Gly-Pro, a central domain lacking acidic residues but enriched in Ser-Thr and, finally, a C-terminal domain enriched in Arg [8]. The secondary structure of these peptides in aqueous solution is unstructured as proposed by von JelLINE [9], but in membranes enriched they adopt mainly α -helix structure [10,11]. Several works have shown that transit peptides have a preference for aromatic and/or typical organelle lipids over mitochondrial and chloroplastic lipids [11-15]. To date the stability of these peptides at the interface between mitochondria and chloroplast [11-15].

INTRODUCTION

Abstract: The surface properties of pure RuBisCo transstil peptide (RTT) and its interaction with zwitterionic, anionic phospholipids and chloroplast lipids were studied by using the Langmuir monolayer technique. Pure RTT is able to form insoluble films and the observed surface parameters are comparable with an α -helix pentamericular to the interface. The α -helix structure tendency was also observed by FT-IR spectroscopy in bulk system of a membrane mimicking environment (SDS). On the other hand, RTT adopts an unordered structure in either aqueous free interface or in the presence of vesicles composed of a zwitterionic phospholipid (POPC). Monolayer studies show that in peptide/lipid mixed monolayers, RTT shows no interaction with zwitterionic phospholipids, regardless of their physical state. Also, with the amionic POPC at high peptide ratios RTT retains its individual surface properties as an immiscible component of the peptide/lipid mixed interface. This behavior was also observed when the mixed films were composed by RTT and the typical chloroplast lipids MGCG or DGDG (mono- and di-galactosyldiacylglycerol). Conversely, RTT establishes a particular interaction with phosphatidylglycerol and cardiolipin at low peptide to lipid area covered ratio. This interaction takes place with an increase in surface stability and a reduction in peptide molecular orientation. Data suggest a dynamic membrane modulation by which the peptide fine-tunes its membrane orientation and its lateral stability, depending on the quality (lipid composition) of the interface. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Published 25 October 2006; revised 11 December 2006; accepted 21 December 2006

University College London Hospital, Grafton Way, London WC1E 6BT, UK

ERNESTO E. AMBROGGIO, ^a BRIAN AUSTEN^b and GERARDO D. FIDELIO^a

Bioophysical properties of a synthetic transit peptide from wheat chloroplast ribulose 1,5-bisphosphate carboxylase

To date the stability of these peptides at the interface is unknown, and whether this stability depends on the structure that peptides may adopt at the amphiphilic interface. Nothing is known about the lateral interaction of these peptides with lipids and whether the peptide-lipid interaction depends on the physical state of lipid phase. In order to correlate protein transport across membranes with the peptide

Keywords: chloroplasts; transplastid peptide; peptide lipid interaction; peptide lateral surface stability; language model layers; LTR

Abstract: The surface properties of pure Rubisco transst peptide (RTP) and its interaction with zwitterionic, anionic phospholipids and chloroplast lipids were studied by using the Langmuir monolayer technique. Pure RTP is able to form insoluble films and desorbed surface parameters are comparable with an a-helix perpendicular to the interface. The a-helix structure tendency also observed by using transmission FT-IR spectroscopy in bulk system of a membrane mimicking environment (SIS). On other hand, RTP adopts an undirected structure in either aqueous free interface or in the presence of vesicles composed of zwitterionic phospholipid (POPC). Monolayer studies show that in peptide/lipid mixed monolayers, RTP shows no interaction with zwitterionic phospholipids, regardless of their physical state. Also, with the anionic POPG at high peptide ratios RTP retains individual surface properties and behaves as an immiscible component of the peptide/lipid mixed interface. This behavior is also observed when the mixed films were composed by RTP and the typical chloroplast lipids MGCG or DGDG (mono- and galactosyldiacylglycerol). Conversely, RTP establishes a particular interaction with phosphatidylglycerol at low peptide to lipid area coverage relation. This interaction takes place with an increase in surface stability and a reduction in peptide molecular area (intermolecular interaction). Data suggest a dynamic membrane modulation by which the peptide fine-tunes its membrane orientation and its lateral stability, depending on the quality (lipid composition) of the interface. Copyright © 2007

Published 25 October 2006; Revised 11 December 2006; Accepted 21 December 2006

CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, Haydá de la Torre y Medina Allende, Ciudad Universitaria, 5000 Córdoba, Argentina

Bioophysical properties of a synthetic transiti peptide from wheat chloroplast ribulose 1,5-bisphosphate carboxylase

secondary structure, the peptide surface activity and peptide-lipid lateral interaction, we have studied the biophysical properties of the first 24 residues from wheat RTP [16]. Briefly, in this study we analyse the surface behaviour of pure peptide and mixed peptide-lipid films by using the air-water monolayers approach as a membrane model system. Also, we include structural analysis of RTP provided by FT-IR in different biomembrane-like environments. Altogether, we conclude that RTP adopts α -helical conformation at the air-water interface and in bulk when the peptide interacts with SDS. Also, differential lateral interactions of the peptide with lipids were observed for peptide/lipid mixed films at the air-water interface, depending on the interfacial components in the mixture.

MATERIALS AND METHODS

Materials

Lipids (POPC, DPPC, POPG and Cardiolipin) were purchased from Avanti Polar Lipids Co. (Alabaster, AL, USA). Cardiolipin is an extracted lipid from beef heart tissue and the main fatty acid composition is 18:3 (86.6%). MGDG and DGDG were obtained from Lardan fine chemicals (Malmö, Sweden). All lipids were used without further purification. $^2\text{H}_2\text{O}$ and SDS were provided Sigma-Aldrich Chem. Co. (St. Louis, MO, USA).

The 24 residue RTP peptide: Met-Ala-Pro-Ala-Val-Met-Ala-Ser-Ser-Ala-Thr-Thr-Val-Ala-Pro-Phe-Gln-Gly-Leu-Lys-Ser-Thr-Ala-Gly was synthesized on *p*-alkoxybenzylpolystyrene with fluorescamine-protected amino acids on a Milligen 9050 Peptide Synthesizer using the protocol described by the manufacturers, cleaved and deprotected by incubation in 50% TFA, 5% anisole, 2.5% ethyl methyl sulphide and 42.5% dichloromethane for 2 h, triturated in diethyl ether, then purified by reverse-phase HPLC on a C8 Rainin column.

Transmission infrared studies

FT-IR spectra of RTP (6.7 mg/ml) in $^2\text{H}_2\text{O}$ and in presence of 15% SDS, POPC and POPG dispersions in $^2\text{H}_2\text{O}$ were recorded 12 h after sample preparation (to ensure a complete H/D exchange) on a Nicolet Nexus spectrometer, at room temperature in a CaF_2 cell with a 0.1 μm Teflon spacer, continuously purged with dry air to eliminate water vapour interference. One hundred scans were signal-averaged at a resolution of 2 cm^{-1} . Before FT-IR measurements, RTP peptide was lyophilized several times from 10 mM HCl in order to eliminate traces of TFA. Spectra of peptide-free samples were subtracted from the spectra of the RTP-containing samples, using OMNIC E.S.P. 5.1 software. Fourier self-deconvolution was performed and the values for the bandwidth and the enhancement for the deconvoluted spectra were 18 and 2 cm^{-1} , respectively [17].

Monolayer studies

The Monolayer technique is a useful technique in order to study amphiphatic molecules at the air-liquid interface. It is based on the direct measurement of changes in the surface

tension of the liquid (subphase), when a surface compressed by a barrier. This change in surface translated to Π where $\Pi = \gamma_0 - \gamma$, γ_0 being the surface tension of the pure liquid and γ , the tension of the film-covered by the barrier [18]. Monolayer experiments were performed at room temperature, ($25 \pm 2^\circ\text{C}$). The subphase was 145 mM NaCl. Lipids were dissolved in chloroform (67:33, v/v) solution. Pure peptide monolayers were prepared by direct spreading from DMSO: chloroform:methanol (1:1, v/v) solution (1 mM) by using a microsyringe. For each experiment, the total surface area of the Teflon trough was 80 cm^2 and the volume of the subphase was 75 μl at the specified pH. The spreading solvent was allowed to stand for at least 5 min before compression was started at a rate of $43\text{ cm}^2/\text{min}$. Lower compression rates gave similar results. For lipid-peptide mixed monolayers, peptides were premixed at the desired proportion from their respective pure solutions, and then directly spread on the subphase. The Π (Wilhelmy method via platinized-Pt plate), ΔV (via millivoltmeter), Π (via air-ionizing ^{241}Am plate and calomel electrode pair) were automatically measured (with the control unit Monofil 2000, Film Lift, Mayer Feintechnik, Göttingen, Germany). Π and ΔV were recorded continuously and simultaneously with a channel X-Y recorder.

RESULTS

FT-IR analysis

For peptide secondary structure, the amide I band (placed between 1615 and 1695 cm^{-1} at the 1648 cm^{-1} maximum) was analysed [19,20]. At this specific region of the FT-IR spectrum, the absorption (vibration) of the carbonyl group of the amide bond is analysed. The amide group absorbs at typical wavenumbers of the spectrum, depending on the secondary structure it is conforming [19]. The amide I band of the deconvolved spectra of the 24-residue synthetic peptide in $^2\text{H}_2\text{O}$, SDS micelles and POPC vesicles are shown in Figure 1(A). For a more detailed analysis of the amide I band, the second derivative spectra of the deconvolved spectrum was generated (Figure 1B). The second derivative spectra of RTP in $^2\text{H}_2\text{O}$ and in the presence of POPC vesicles show mainly absorption bands in the 1642 – 1644 cm^{-1} frequency range, which are indicative of a random structure. Small absorption bands at 1626 cm^{-1} (associated with β -sheet structure) can be observed probably due to some peptide aggregation [20,21].

It is possible to notice a difference between the amide I maximum in the deconvolved spectra of RTP in 15% SDS, centred at 1648 cm^{-1} , with respect to that obtained in either spectra of pure RTP in $^2\text{H}_2\text{O}$ or in the presence of POPC vesicles, which is centred at 1643 cm^{-1} (Figure 1(A)). The 1647 – 1650 cm^{-1} wavenumber range is associated to an α -helical structure, when $^2\text{H}_2\text{O}$ is used as solvent [19,20].

Strato
synthe
propri

- High
- Basic
- Technical
- Commercial

From
for AP

- Biological
- Chemical
- Biotechnology
- Instrumentation

The lower collapse pressure corresponds to a peptide-enriched phase, whereas the higher collapse pressure corresponds to a more lipid-enriched phase [23,24].

Mixed films of DPPC and POPC show an immiscible behavior at all peptide-lipid proportions. This can be deduced from T-A isotherms in which two collapse pressures are clearly distinguishable (Figure 3) according to the surface phase rule, refer [18,22].

$$A_{\text{equivalent}} = (A_1 \times A_2) / X_1 \quad (2)$$

$$A_{ideal}[n] = A_{lipid}[n] \times X_{lipid} + A_{peptide}[n] \times X_{peptide} \quad (1)$$

The monolayer technique is a powerful approach to study the surface properties of amphiphatic molecules either when constituting a one-component film or when interacting with other amphiphilic molecules at the interface. This technique allows to know the precise interfacial composition: to control the lateral packing of the film; and to adjust external variables like pH, temperature, etc. From mixed lipid-peptide monolayers it is possible to obtain the exact mean molecular area (A_{m}) of the mixture and, therefore, it is possible to compare them with the ideal area (A_{id}), which is the expected area from a non-interacting mixture. This data allows us to know the lateral interaction parameter π that is calculated from the difference between the measured area (A_{m}) of the mixture and the ideal area (A_{id}). The idea of repulsive forces between the components in the mixture is calculated from the lateral pressure (Eqn 1).

$$\pi = \frac{A_{\text{id}} - A_{\text{m}}}{A_{\text{id}}}$$

For pure components weighted by their respective mole fraction at the desirous lateral pressure (Eqn 1, 18,22-25). Densities from the additive rule indicate also possible to estimate the equivalent area ($A_{\text{equivalent}}$) of the peptide in the mixed interphase according to Eqn 2 by assuming that the molecular lipid area remains unchanged at the evaluated lateral pressure [18,22].

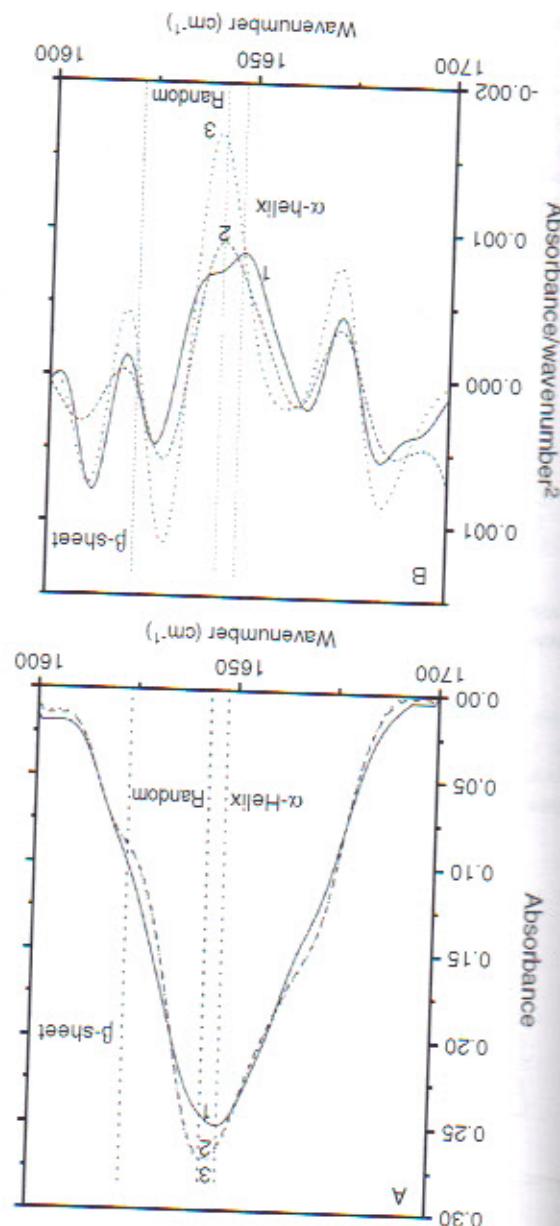
Lipid-peptide mixed monolayers

These three surface parameters are closely similar to those found for the amphiphilic multilin α -helices (23,24), in agreement with an α -helix structure per peptide (23,24), in the amorphophilic multilin α -helical peptides (23,24), in the amorphophilic multilin α -helices found for the amorphophilic multilin α -helices (23,24). The values of collapse pressure and limiting molecular area of RPT are independent of the pH of the subphase. However, under alkaline or acidic subphase the UV changes owing to the titration of lateral amino groups, which, in turn, modifies the net dipolar moment across the interface (Figure 2, refer [18,22] for further details on AV). This analysis is relevant in order to note whether changes in the pH of the interface (quality) induce changes in peptide conformation affecting its surface behaviour.

two-dimensional array of the metrifice, refer [18,22]. At maximum packing (collapse) pressure of 2.16 nm^{-2} per molecule, with a collapse pressure of 17 MN m^{-1} (Figure 2), The ΔV of the peptide monolayer at these surface pressures was 365 mV .

Pure RTP peptide forms insoluble monolayers giving a limiting molecular area (maximum packing in the suspension in aqueous environment).

Figure 1 Transmission FT-IR spectra of RTR in different H_2O solution (3). The peptide concentration is 6.7 mg/ml.



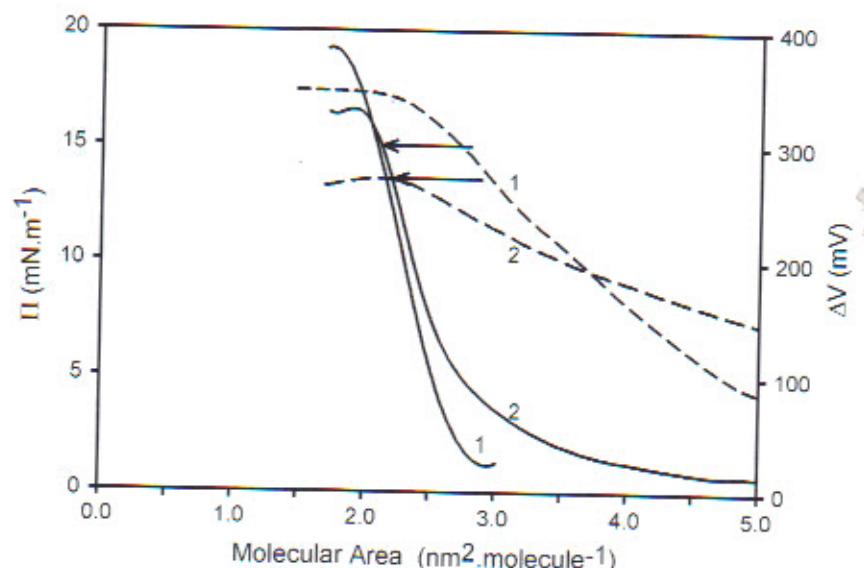


Figure 2 Surface behaviour of pure RTP peptide monolayers at different pH subphase. Π -Area (solid line) and ΔV -Area (dashed line) isotherms of RTP monolayers on a subphase of 145 mM NaCl at pH 6 (1) or 11 (2). Arrows indicate the collapse pressure of monolayers.

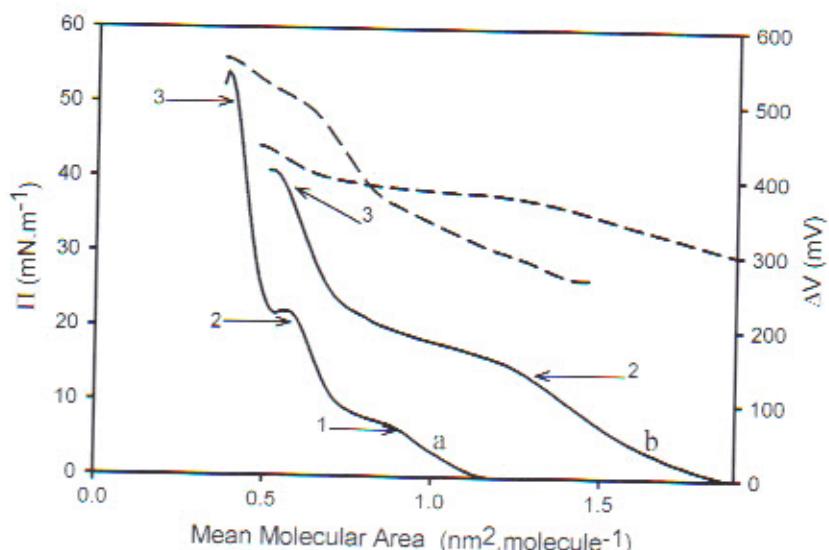


Figure 3 Π -Area compression isotherms of RTP-zwitterionic lipid mixed monolayers at similar area covered peptide. Π -Area (solid line) and ΔV -Area (dashed line) of RTP/DPPC at 0.06:0.94 mole ratio (or 25:75% area ratio) (a) and RTP/POPC at 0.24:0.74 mole ratio (or 75:25% area ratio) (b). The subphase was NaCl 145 mM at pH 6. Arrows 1: Liquid expanded to liquid condensed phase transition of DPPC distorted by the presence of the peptide; 2: Collapse pressure of the peptide-enriched phase; 3: Collapse pressure of the lipid-enriched phase.

No considerable deviations were observed in the mean molecular area compared with the expected ideal behaviour (Eqn 1, data not shown). Thus, no substantial lateral peptide-lipid interactions are observed with zwitterionic phospholipids. The mean ΔV and the mean molecular area values found for RTP mixed with neutral phospholipids are indicative that the peptide retains its α -helical conformation perpendicular to the interface.

Compression isotherms of monolayers composed by RTP mixed with the typical chloroplast lipids MGDG and DGDG were analysed. As seen in Figure 4(A)

and (B), RTP is not miscible with these lipids at all peptide proportions studied (two collapse pressures are observed at the isotherm).

Conversely, RTP shows a miscible behaviour in mixed films with anionic lipids like POPG or cardiolipin up to a 1:3 peptide-lipid area relation (Figure 5). A further increase in peptide area proportion produces an immiscible behaviour (Figure 5). Interestingly, in the range of miscibility of negative peptide-lipid interfaces the equivalent peptide area is substantially reduced (Table 1).

peroxisome [27, 28]. In chloroplasts, the import of nucleic-encoded proteins is mediated by the interac-
tion between the mitochondrial *N*-terminal translat-
ion and a common import machinery of the preproteins [1, 15]. One of the general characteristics of signalling peptides that is emerging is the general amphiphilic nature of the signal sequence [6, 7]. Thus, in the insertion of translocation into biomembranes the rather than an exact sequence matching [6, 7]. This is peptide-lipid interaction acquires relevance since it is the integral secretory signal sequence peptides adopt β -sheet structure both in aqueous solutions and in presence of phospholipids and detergents and, in turn, this conformation is responsible for the high surface sta-
bility acquired for these peptides at air-water interface [19, 29, 30]. In contrast, the FT-IR spectra of the 24-
residue chloroplastic RFP either in aqueous solution or
in presence of zwitterionic interfacial solution essentia-
lly agree with those reported for the high surface sta-
bility of the 24-residue chloroplastic RFP [19, 29, 30]. In contrast, the FT-IR spectra of the 24-
residue chloroplastic RFP either in aqueous solution or
in presence of zwitterionic interfacial solution essentia-
lly agree with those reported for the high surface sta-
bility of the 24-residue chloroplastic RFP [19, 29, 30].

Cells have mechanisms that differentiate the signal sequences in the newly synthesized proteins that later translocate secretory and membrane proteins into the endoplasmic reticulum membrane. Also, the apoproteins imported into chloroplasts are cleaved into chloroplasts. Additionally, the nucleus or other subcellular organelles such as the nucleus import proteins into mitochondria from those that direct proteins to other organelles such as the nucleus or the endoplasmic reticulum.

DISCUSSION

Figure 6. Changes in membrane fluidity of PC/POG mixed with POG and saturated fatty acids at 0.05:0.95 (A) and 0.21:0.79 (B) molar ratios. The effect of palmitic acid on the membrane fluidity was measured by the change in the area of the peptide–lipid complex (ΔA) and the change in the area of the peptide–lipid complex (ΔV) in the presence of palmitic acid compared to the control (no palmitic acid). The palmitic acid concentration was varied from 0 to 10 mol %.

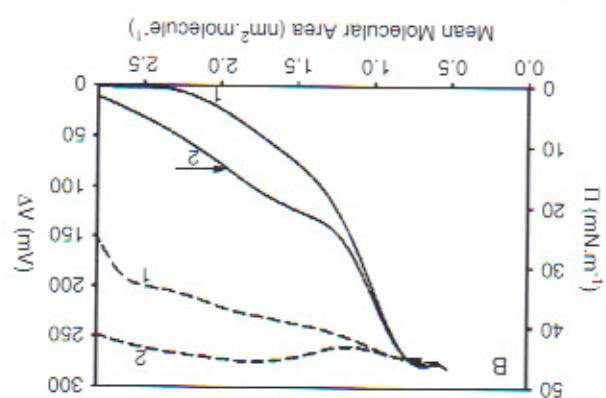
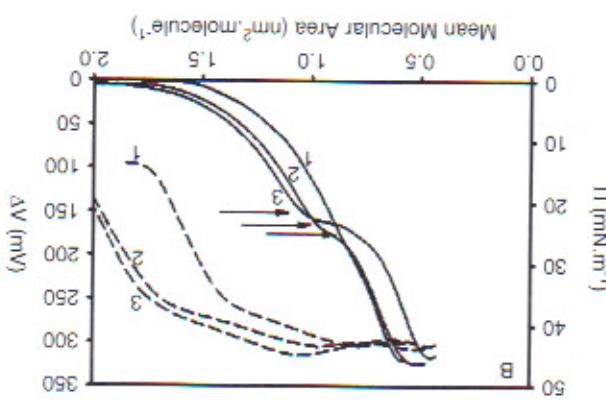


Figure 5. Circular dichroism measurements of RTT-DGCG peptide-lipid mixed monolayers at peptide to lipid mole ratio of 0.03:0.97 (corresponding to 10:90 peptide:lipid covered area) (curve 1), 0.09:0.91 (corresponding to 23:77 peptide:lipid covered area; curve 2) and 0.23:0.77 (corresponding to 50:50 peptide:lipid covered area; curve 3). Arrows: collapse pressure of the peptide enriched phase (immissibility condition).



CHLOROPLAS T TRANSIT PEPTIDE-UPID INTERACTION

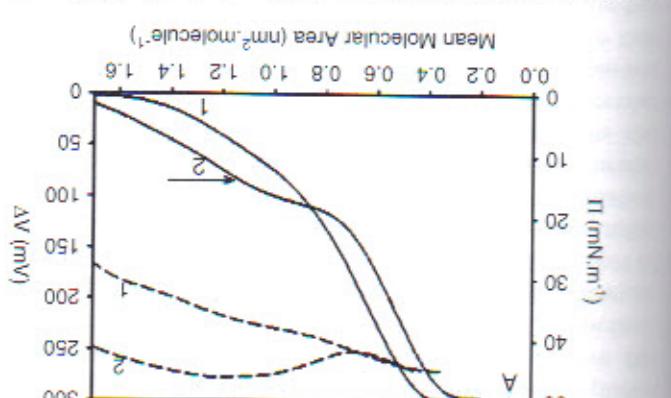


Table 1 Equivalent molecular area of RTP peptide in different condition

Monolayer composition (lipid/RTP mole ratio)	Equivalent molecular area ^a (nm ² molecule ⁻¹)	Mixing behaviour observed
Pure RTP Peptide	2.16	—
RTP/DPPC (0.17/0.83)	2.38	Immiscible
RTP/POPC (0.24/0.76)	2.46	Immiscible
RTP/POPG (0.05/0.95)	1.07	Miscible
RTP/POPG (0.24/0.86)	2.00	Immiscible
RTP/cardiolipin (0.10/0.90)	1.05	Miscible
RTP/cardiolipin (0.22/0.78)	3.18	Immiscible
RTP/MGDG (0.77/0.23)	—	—
(0.91/0.09)	1.7	—
(0.97/0.03)	2.65	Immiscible
RTP/DGDG (0.77/0.23)	—	—
(0.91/0.09)	2.83	—
(0.97/0.03)	1.84	—
RTP/(MGDG- POPG 8:2) (0.77/0.23)	—	—
(0.91/0.09)	2.37	Immiscible
(0.97/0.03)	1.5	—
RTP/(DGDG- POPG 8:2) (0.77/0.23)	—	—
(0.91/0.09)	2.21	—
(0.97/0.03)	2.73	—
RTP/(MGDG- POPG 8:2) (0.77/0.23)	2.3	—
(0.91/0.09)	2.48	—
(0.97/0.03)	3.15	Immiscible
	3.99	—

^a The equivalent molecular area was calculated according to Eqn 2.

random structure. However, RTP when interacting with SDS micelles adopts mainly an α -helical conformation (Figure 1). This finding is in keeping with the hypothesis presented by von Heijne *et al.*, [9] in which they postulate that the chloroplast transit peptide sequences are compatible with a more flexible conformation. Furthermore, it was reported that both the rat malate dehydrogenase mitochondrial transit peptide or the preferredoxin chloroplast transit peptide have random coil conformation in water solution [11,31]. However, for all the peptides indicated above they increase the amount of secondary α -helix structure in presence of particular amphiphilic membrane mimic systems. Empirical calculations have suggested that the common structural feature of mitochondrial transit sequences is the amphiphilic helix [32,33].

The present paper is the first in describing the surface stability and peptide-lipid lateral miscibility of mixed films at air-water interface corresponding to a well-defined 'transit peptide'. RTP forms insoluble monolayers with a molecular area of about 2.16 nm² at a collapse pressure of 17 mN m⁻¹ (Figure 2). This data is similar to that obtained for the lytic 26-residue melittin or antibiotic peptides, which is compatible with a α -helix structure perpendicular to the interface [23,24]. The unmaximal stability of RTP of about 17 mN m⁻¹ is relatively low and scores below what is expected for a phospholipid in a bilayer (around 30–35 mN m⁻¹, [34,35]) or for signal sequence peptides (higher than 25 mN m⁻¹, [29,30]). Pure RTP monolayer has a low collapse pressure compared to other peptides that adopt β -sheet structure at the interface [28,29,36–38]. The lower stability of α -helix peptides at the interface may be because this structure has a low degree of lateral interaction, in contrast with peptides that adopt β -sheet structure in which they have the possibility of lateral inter peptide-peptide interaction, typical of the β -sheet conformation [36]. In addition, we have measured the surface properties at two pH of the subphase. At the alkaline pH, the basic amino groups of the peptide sequence may be deprotonated. This change is noticed by lateral ΔV measurements. Here, we report that no noticeable changes in the surface behaviour (collapse pressure and molecular area of the peptide film) are observed, whereas a marked change in the ΔV was noticed. This data indicate that there is a change in the net dipole of the molecule but this change is not caused by a change in the protein structure [24].

The difference in peptide conformation and surface stability may, in turn, be influencing the lateral peptide-lipid miscibility [23,25,29]. The RTP-lipid miscibility depends on lipid polar head group of the phospholipid. RTP peptide, which does not discriminate the physical state of phospholipids, since with the zwitterionic DPPC or POPC we found an immiscible behaviour in all peptide-lipid mixed interface composition (Figure 3); these lipids behave as liquid condensed and liquid-expanded monolayer phase, respectively, at room temperature [22,23]. Also, an immiscible behaviour was observed for the chloroplast component lipids, MGDG and DGDG. On the other hand, RTP is miscible either with the anionic POPG or cardiolipin up to 25% of peptide relative area proportion (it corresponds to a peptide mole fraction of about 0.05; Table 1). In the mixed surface behaviour, the relative area of the components acquires more importance than the mole fraction if the difference on the surface molecular area is high [39]. A further increase in peptide content at the mixed interface shows immiscibility with two well-defined collapse pressures (Figure 4). It is interesting to emphasize that in the range of peptide-anionic lipid composition in which a miscible

to terms with membranes. *Biochim. Biophys. Acta* 1988; **947**: 307-333.

¹⁶ von Helmuth G., *Transcending the Impenetrable: how prototypes come into play*, *Journal of Computer Information Systems*, vol. 49, no. 3, pp. 293-313.

role of the trinitol peptide in the routing of precursors toward different chloroplast compartments. Cell 1986; 48: 365-375.

Targeting sequnces: common themes in diverse systems. Mol Membr Biol 1995; 12: 295-307.

4. Rausch SL, Kentala D, Protein transport via amino-terminal

precursor of small subunit of ribulose bisphosphate carboxylase is processed to the mature size in two steps. Eur. J. Biochem. 1984.

22. Agarababtires F.A., Dicic J.R.: Protein translocation across membranes. *Biochim. Biophys. Acta*, 2001; **1513**: 1-24.

23. Robinsom C., Elliss R.J.: Transport of proteins into chloroplasts. *The Biochemical Journal*, 2001; **356**: 1-12.

11. Chen X, Schmidl DL. Protein import into chloroplasts. *Trends Cell Biol* 1999; 9: 222-227.

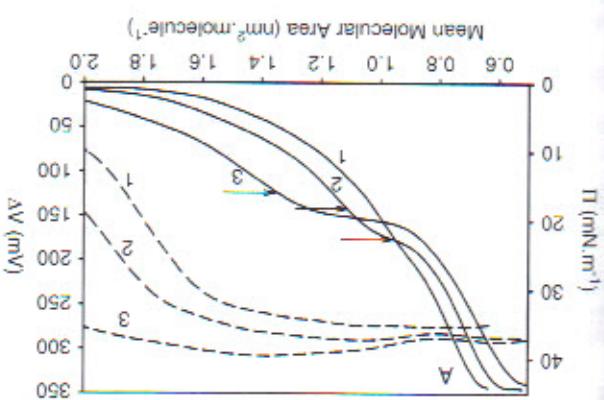
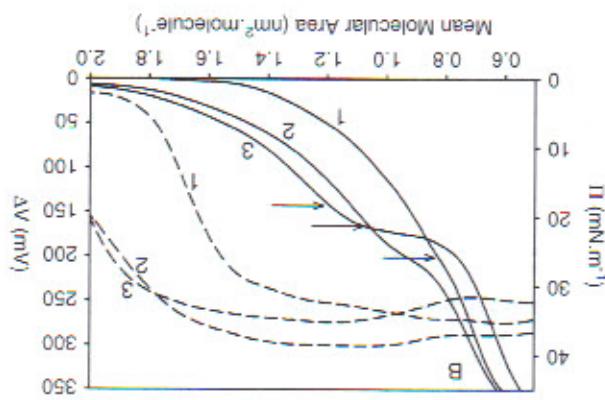
REFERENCES

REFERENCES

This work was supported by grants from CONICET, FONCYT (PICT 0609228), SECYT-UNC, and Agencia Córdoba Ciencia. G.D.F., SECTY-UNC, and Agencia FONCYT (PICT 0609228), SECYT-UNC, and St. George's Hospital, Royal Free Hospital and St. George's University.

The fact that RTP is not miscible with zwitterionic lipids at the air/water interface, this can be an explanation of why it cannot interact with POPC liposomes in bulk (the mean lateral pressure assumed for liposomes in bulk [34,35], with all these data, it is noticeable that a fine-tune regulation for the protein transport at the peptide/lipid interaction level can be involved for this process).

Figure 6 μ -WIRE compression isotherms of KIT-1/DGCG/POPG (0.8-0.2) (A) and KIT-1/DGCG/POPG (0.8-0.2) mole ratio lipid/POPG 0.8:0.2 mixed monolayers.



7. Robinson A, Austen B. The role of topogenic sequences in the movement of proteins through membranes. *Biochem J* 1987; **246**: 249–261.
8. Bruce BD. Chloroplast transit peptides: structure, function and evolution. *Trends Cell Biol* 2000; **10**: 440–447.
9. von Heijne G, Nishikawa K. Chloroplast transit peptides. The perfect random coil? *FEBS Lett* 1991; **278**: 1–3.
10. Wienk HL, Czisch M, de Kruijff B. The structural flexibility of the preferreddoxin transit peptide. *FEBS Lett* 1999; **453**: 318–326.
11. Wienk HL, Wechselberger RW, Czisch M, de Kruijff B. Structure, dynamics, and insertion of a chloroplast targeting peptide in mixed micelles. *Biochemistry* 2000; **39**: 8219–8227.
12. van 't Hof R, Demel RA, Keegstra K, de Kruijff B. Lipid-peptide interactions between fragments of the transit peptide of ribulose-1,5-bisphosphate carboxylase/oxygenase and chloroplast membrane lipids. *FEBS Lett* 1991; **291**: 350–354.
13. van 't Hof R, van Klompenburg W, Pilon M, Kozubek A, de Korte-Kool G, Demel RA, Weisbeek PJ, de Kruijff B. The transit sequence mediates the specific interaction of the precursor of ferredoxin with chloroplast envelope membrane lipids. *J Biol Chem* 1993; **268**: 4037–4042.
14. Leenhouts JM, Tork Z, Demel RA, de Gier J, de Kruijff B. The full length of a mitochondrial presequence is required for efficient monolayer insertion and interbilayer contact formation. *Mol Membr Biol* 1994; **11**: 159–164.
15. Bruce BD. The paradox of plastid transit peptides: conservation of function despite divergence in primary structure. *Biochim Biophys Acta* 2001; **1541**: 2–21.
16. Broglie R, Coruzzi G, Lamppa G, Kieth B, Chua N-H. Structural analysis of nuclear genes coding for the precursor to the small subunit of wheat ribulose-1,5-bisphosphate carboxylase. *Biotechnology* 1983; **1**: 55–61.
17. Ambroggio EE, Villarreal MA, Montich GG, Rijkers DT, De Planque MR, Separovic F, Fidelio GD. Interfacial properties of the M1 segment of the nicotinic acetylcholine receptor. *Biophys Chem* 2006; **121**: 171–176.
18. Gaines GL. *Insoluble Monolayers at Liquid-gas Interfaces*. Interscience: New York, Prigogine, 1966.
19. Arondo JL, Muga A, Castresana J, Goni FM. Quantitative studies of the structure of proteins in solution by Fourier transform infrared spectroscopy. *Prog Biophys Mol Biol* 1993; **59**: 23–56.
20. Haris PI, Chapman D. Does Fourier-transform infrared spectroscopy provide useful information on protein structures? *Trends Biochem Sci* 1992; **9**: 328–333.
21. Haris PI, Fidelio GD, Austen BM, Lucy JA, Chapman D. Secondary structure of signal sequence peptides in the presence and absence of lipids: a Fourier transformed infrared spectroscopy investigation. *Biochem Soc Trans* 1987; **15**: 1129–1131.
22. Birdi KS. *Lipid and Biopolymer Monolayers at Liquid Interfaces*. Plenum Press: New York, 1989.
23. Fidelio GD, Maggio B, Cumar FA. Interaction of melittin with glycosphingolipids and phospholipids in mixed monolayers at different temperatures. Effect of the lipid physical state. *Biochim Biophys Acta* 1986; **862**: 49–56.
24. Ambroggio EE, Separovic F, Bowie J, Fidelio GD. Surface behaviour and peptide-lipid interactions of the antibiotic peptides, Maculatin and Citropin. *Biochim Biophys Acta* 2004; **1664**: 31–37.
25. Maget-Dana R. The monolayer technique: a potent tool for studying the interfacial properties of antimicrobial and membrane-lytic peptides and their interactions with lipid membranes. *Biochim Biophys Acta* 1999; **1462**: 109–140.
26. Pinnaduwage P, Bruce BD. In vitro interaction between a chloroplast transit peptide and chloroplast outer envelope lipids is sequence-specific and lipid class-dependent. *J Biol Chem* 1996; **271**: 32907–32915.
27. Paschen SA, Neupert W. Protein import into mitochondria. *J Membr Life* 2001; **52**: 101–112.
28. Emanuelsson O, von Heijne G. Prediction of organellar targeting signals. *Biochim Biophys Acta* 2001; **1541**: 114–119.
29. Fidelio GD, Austen BM, Chapman D, Lucy JA. Interaction of ovoalbumin and its putative signal sequence with phospholipids. Possible importance of differing lateral stabilities in protein translocation. *Biochem J* 1987; **244**: 295–301.
30. Fidelio GD, Austen BM, Chapman D, Lucy JA. Properties of signal sequence peptides at an air-water interface. *Biochem J* 1986; **238**: 301–304.
31. MacLachlan LK, Haris PI, Reid DG, White J, Chapman D, Lucy JA, Austen BM. A spectroscopic study of the mitochondrial transit peptide of rat malate dehydrogenase. *Biochem J* 1994; **303**: 657–662.
32. von Heijne G. Mitochondrial targeting sequences may form amphiphilic helices. *EMBO J* 1986; **6**: 1335–1342.
33. Karlin-Neumann GA, Tobin EM. Transit peptides of nuclear-encoded chloroplast proteins share a common amino acid framework. *EMBO J* 1986; **1**: 9–13.
34. Mohwald H. Phospholipid and phospholipid-protein monolayers at the air/water interface. *Annu Rev Phys Chem* 1990; **42**: 441–476.
35. Feng SS. Interpretation of mechanochemical properties of lipid bilayer vesicles from the equation of state or pressure-area measurement of the monolayer at the air-water or oil-water interface. *Langmuir* 1999; **15**: 998–1010.
36. Maget-Dana R, Lelièvre D, Brack A. Surface active properties of amphiphilic sequential isopeptides: Comparison between alpha-helical and beta-sheet conformations. *Biopolymers* 1999; **40**: 415–423.
37. Ambroggio EE, Kim DH, Separovic F, Barrow CJ, Barnham KJ, Bagatelli LA, Fidelio GD. Surface behavior and lipid interaction of Alzheimer beta-amyloid peptide 1–42: a membrane-disrupting peptide. *Biophys J* 2005; **88**: 2706–2713.
38. Lau TL, Ambroggio EE, Tew DJ, Cappai R, Masters CL, Fidelio GD, Barnham KJ, Separovic F. Amyloid-beta peptide disruption of lipid membranes and the effect of metal ions. *J Mol Biol* 2006; **358**: 759–770.
39. Fidelio GD, Maggio B, Cumar FA. Interaction of myelin basic protein, melittin and bovine serum albumin with gangliosides sulphatide and neutral glycosphingolipids in mixed monolayers. *Chem Phys Lipids* 1984; **35**: 231–245.