Journal of Peptide Science
J. Pept. Sci. 2007; **13**: 245–252
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.838

JOURNAL OF
PEPTIDE SCIENCE

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

ERNESTO E. AMBROGGIO,[a] BRIAN AUSTEN[b] and GERARDO D. FIDELIO[a]*

[a] CIQUIBIC, CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5000-Córdoba, Argentina
[b] Neurodegeneration Unit, Department of Surgery, St.George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 ORE, UK

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

Abstract: The surface properties of pure RuBisCo transit 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 the observed surface parameters are compatible with an α-helix perpendicular to the interface. The α-helix structure tendency was also observed by using transmission FT-IR spectroscopy in bulk system of a membrane mimicking environment (SDS). On the other hand, RTP 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, RTP shows no interaction with zwitterionic phospholipids, regardless of their physical state. Also, with the anionic POPG at high peptide ratios RTP retains its individual surface properties and behaves as an immiscible component of the peptide/lipid mixed interface. This behaviour was also observed when the mixed films were composed by RTP and the typical chloroplast lipids MGDG or DGDG (mono- and di-galactosyldiacylglycerol). Conversely, RTP establishes a particular interaction with phosphatidylglycerol and cardiolipin at low peptide to lipid area covered 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 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: chloroplast transit peptide; peptide-lipid interaction; peptide lateral surface stability; langmuir monolayer; FT-IR; chloroplast lipids

INTRODUCTION

Many chloroplast proteins are known to be synthesized on cytosolic ribosomes and imported into the chloroplast after translation, where they end up in specific locations, either in the envelope membranes, the stroma or the thylakoid membranes of the lumen [1–4]. Precursors of the small subunit of the stromal protein ribulose 1,5-bisphosphate carboxylase (RuBisCo) contain transit peptides of between 44 and 57 residues in length, depending on the species of origin, and these have been shown by genetic manipulation experiments to contain sufficient information to achieve transport into the chloroplast [5]. A number of these proteins are synthesized as precursors, with amino-terminal extensions, which are cleaved in two stages after transport, and the two sections may be functionally responsible for transport to or into different locations within the chloroplast [3,5,6]. They contain limited regions of hydrophobic residues, many hydroxyl residues (threonine and serine), fewer basic residues and no acidic residues [7]. Although stromal-targeting peptides do not have a conserved primary structure, they contain three distinct regions: an uncharged *N*-terminal domain of ~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 Heijne [9], but in membranous environments they adopt mainly α-helix structure [10,11]. Several works have shown that transit peptides have differential ability to insert into lipid monolayers, with a preference for anionic and/or typical organelle lipids from mitochondria and chloroplast [11–15].

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 amphipathic 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

Abbreviations: DPPC, 2 dipalmitoyl-sn-glycero-3-phosphocholine; FT-IR spectroscopy, fourier-transform infrared spectroscopy; MGDG, monogalactosyldiacylglycerol; DGDG, di-galactosyldiacylglycerol; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)]; RTP, RuBisCo transit peptide; SDS, sodium dodecyl sulphate. ΔV, surface potential; Π, surface lateral pressure.

*Correspondence to: Gerardo D. Fidelio, CIQUIBIC, CONICET, Departamento de Química Biológica, Facultad de Ciencias Químicas, Haya de la Torre y Medina Allende, Ciudad Universitaria, 5000-Córdoba, Argentina; e-mail: gfidelio@dqb.fcq.unc.edu.ar

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

WILEY InterScience® DISCOVER SOMETHING GREAT

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 Larodan fine chemicals (Malmo, Sweden). All lipids were used without further purification. ^2H_2O 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 flourenylmethoxycarbonyl-protected amino acids on a Milligen 9050 Peptide Synthesiser 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 ^2H_2O and in presence of 15% SDS, POPC and POPG dispersions in ^2H_2O 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 amphipathic 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 surfactant film is compressed by a barrier. This change in surface tension is translated to Π where $\Pi = \gamma_o - \gamma$, γ_o being the surface tension of the pure liquid and γ, the tension of the film-covered surface compressed by the barrier [18]. Monolayer experiments were performed at room temperature, $(25 \pm 2)\,^\circ$C. The subphase was 145 mM NaCl. Lipids were dissolved in chloroform : methanol (67 : 33, v/v) solution. Pure peptide monolayers were formed by direct spreading from DMSO: chloroform : methanol (1 : 6 : 2, v/v) solution (1 mM) by using a microsyringe. For compression experiments, the total surface area of the Teflon trough was 80 cm^2 and the volume of the subphase was 75 ml at the specified pH. The spreading solvent was allowed to evaporate for at least 5 min before compression was started, at a rate of 43 cm^2/min. Lower compression rates gave the same results. For lipid–peptide mixed monolayers, peptide and lipid were premixed at the desired proportion from their respective pure solutions, and then directly spread on the surface. The Π (Wilhelmy method via platinized-Pt plate), the area enclosing the monolayer, and the ΔV (via milliVoltmeter with air-ionizing ^{241}Am plate and calomel electrode pair) were automatically measured (with the control unit Monofilmmeter with Film Lift, Mayer Feintechnique, Göttingen, Germany). The data were recorded continuously and simultaneously with a double channel X–YY recorder.

RESULTS

FT-IR analysis

For peptide secondary structure, the amide I band (placed between 1615 and 1695 cm^{-1} at the IR spectrum) 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. This group absorbs at typical wavenumbers of the FT-IR spectrum, depending on the secondary structure that is conforming [19]. The amide I band of the FT-IR deconvolved spectra of the 24-residue synthetic transit peptide in ^2H_2O, SDS micelles and POPC vesicles are shown in Figure 1(A). For a more detailed analysis of the amide I band, the second derivative spectrum of the deconvolved spectrum was generated (Figure 1(B)). The second derivative spectra of RTP in ^2H_2O and in presence of POPC vesicles show mainly absorbance bands in the 1642–1644 cm^{-1} frequency range, which are indicative of a random structure. Small absorbance 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 ^2H_2O 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 ^2H_2O is used as solvent [19,20]. These

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

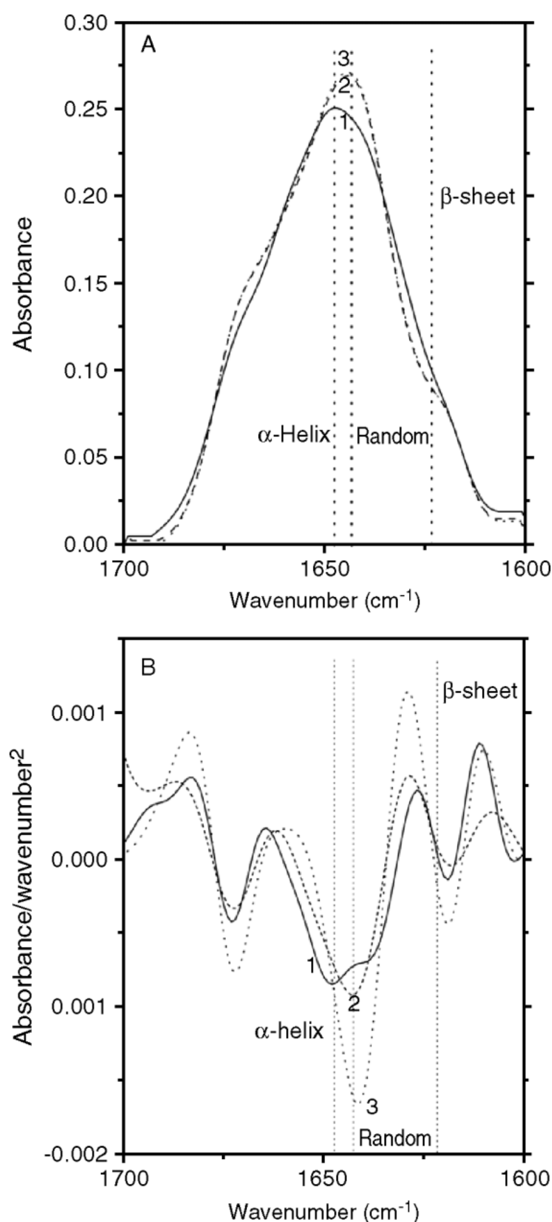

Figure 1 Transmission FT-IR spectra of RTP in different conditions. FT-IR absorbance spectra (A) and second derivative spectra (B) of RTP in 15% of SDS (1), POPC vesicles (2) and ^2H_2O solution (3). The peptide concentration is 6.7 mg/ml.

results indicate that the peptide in aqueous solution, or in presence of POPC (zwitterionic) vesicles, is mainly in an unordered conformation but in presence of negatively charged SDS micelles there is a significant increase on its α-helical content. FT-IR data from RTP interacting with anionic or chloroplast lipids was not possible to achieve because of peptide/lipid aggregation and because chloroplast lipids were problematic to resuspend in aqueous environment.

Pure peptide monolayer

Pure RTP peptide forms insoluble monolayers giving a limiting molecular area (maximal packing in the two-dimensional array of the interface, refer [18,22]) 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 maximal packing (collapse pressure) was 365 mV.

These three surface parameters are closely similar to those found for the amphiphilic melittin or antibiotics α-helical peptides [23,24], in agreement with an α-helix structure perpendicular to the interface [23–25]. The values of collapse pressure and limiting molecular area of RTP are independent of the pH of the subphase. However, under alkaline or acidic subphase the ΔV 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 ΔV). This analysis is relevant in order to note whether changes in the pH of the interface (quality) induce changes in peptide conformation altering its surface behaviour.

Lipid–peptide mixed monolayers

The monolayer technique is a powerful approach to study the surface properties of amphipathic 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 experimental mean molecular area (A_{1-2}) of the mixture and, therefore, it is possible to compare them with the ideal area (A_{ideal}), which is the expected area from a non-interacting mixture. This data allows us to know the lateral interaction (attractive or repulsive) of the components in the mixture. The ideal area is calculated from the additive values obtained for pure components weighted by their respective mole fraction at the desired lateral pressure (Eqn 1, [18,22–25]). Deviations from the additive rule indicate peptide–lipid lateral interactions [18,22,24,25]. It is also possible to estimate the equivalent area ($A_{\mathrm{equivalent}}$) of the peptide in the mixed interface according to Eqn 2 by assuming that the molecular lipid area remains unchanged at the evaluated lateral pressure [18,22].

$$[A_{\mathrm{ideal}}]_{\Pi} = \left[A_{\mathrm{lipid}}\right]_{\Pi} \times X_{\mathrm{lipid}} + \left[A_{\mathrm{peptide}}\right]_{\Pi} \times X_{\mathrm{peptide}} \quad (1)$$

$$\left[A_{equivalent_2}\right]_{\Pi} = ([A_{1-2}]_{\Pi} - [A_1]_{\Pi} \times X_1) \Big/ X_2 \quad (2)$$

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

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

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

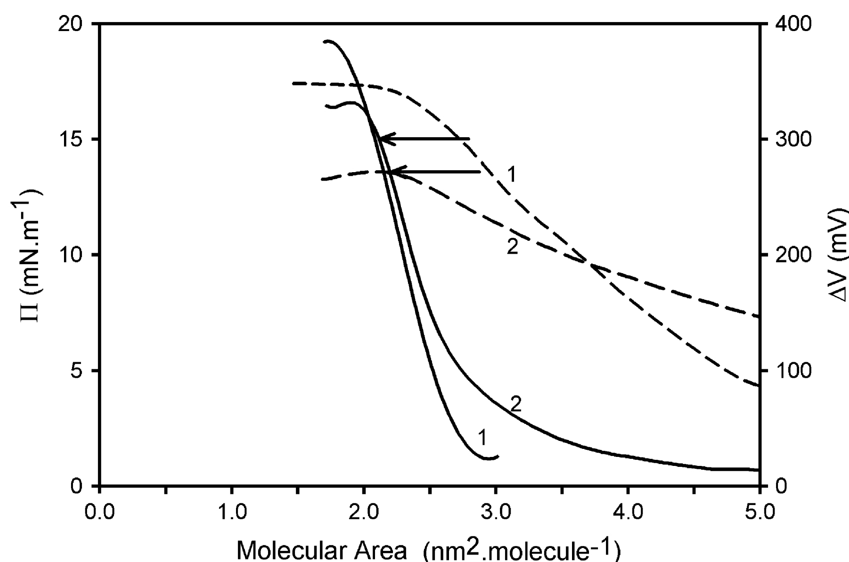

Figure 2 Surface behaviour of pure RTP peptide monolayers at different pH subphase. Π-Area (solid line) and ΔV-Area (dashed line) isotherms of RTP monolayers on a subphase of 145 mM NaCl at pH 6 (1) or 11 (2). Arrows indicate the collapse pressure (Π_c) of monolayers.

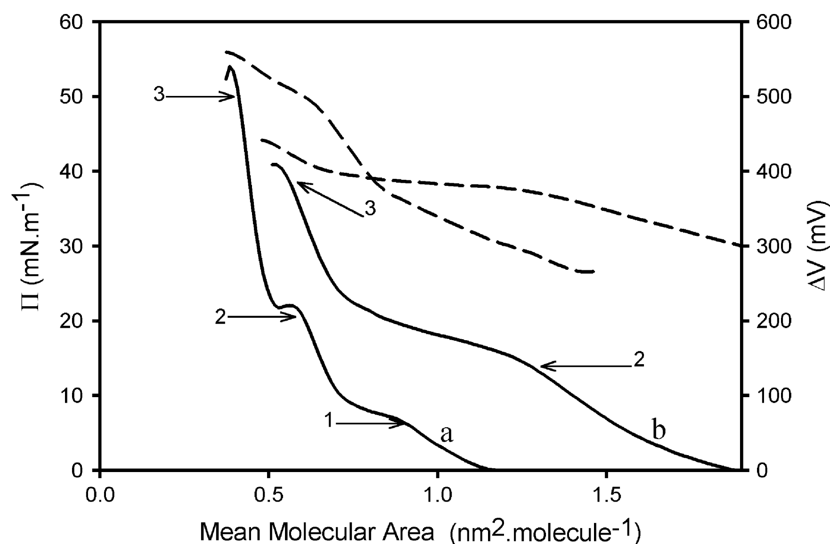

Figure 3 Π-Area compression isotherms of RTP-zwiterionic lipid mixed monolayers at similar area covered peptide. Π-Area (solid line) and ΔV-Area (dashed line) of RTP/DPPC at 0.06 : 0.94 mole ratio (or 25 : 75% area ratio) (a) and RTP/POPC at 0.24 : 0.74 mole ratio (or 75 : 25% area ratio) (b). The subphase 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 where 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).

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

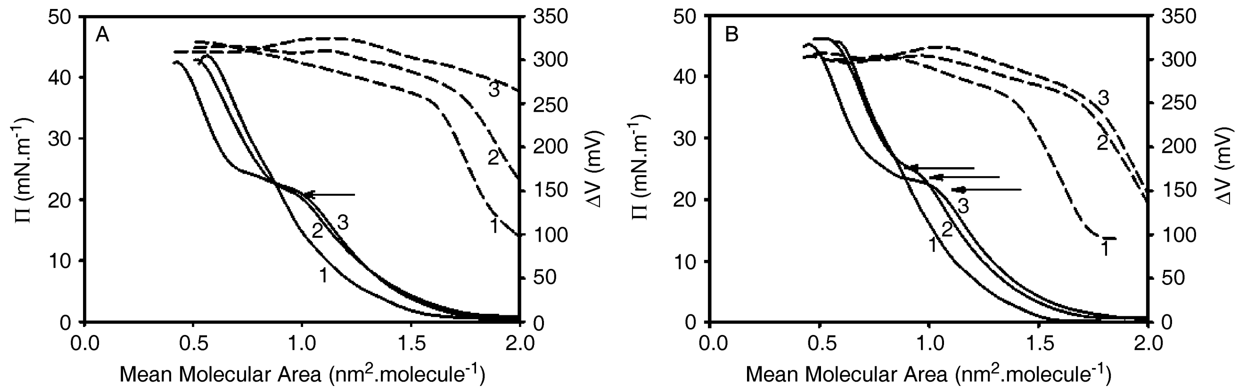

Figure 4 Π-Area compression isotherms of RTP-chloroplast lipids mixed monolayers. RTP-MGDG (A) and RTP-DGDG (B) peptide–lipid mixed monolayers at peptide to lipid mole ratio of 0.03 : 0.97 (corresponding to 10 : 90 peptide : lipid covered area; curve (1), 0.09 : 0.91 (corresponding to 23 : 77 peptide : 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 (immiscibility condition).

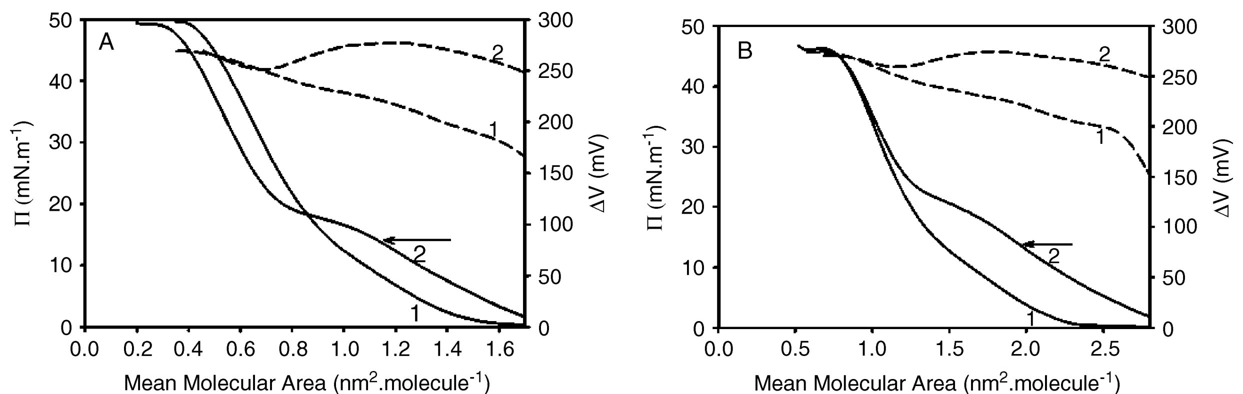

Figure 5 Surface behaviour of RTP mixed with POPG and cardiolipin at similar area covered peptide. Π-Area (solid line) and ΔV-Area (dashed line) of: (A) RTP/POPG at 0.05 : 0.95 peptide : lipid mole ratio (corresponding to 25 : 75 peptide : lipid covered area; curve 1) and at 0.21 : 0.79 peptide : lipid mole ratio (corresponding to 75 : 25 peptide : lipid covered area; curve 2); (B) RTP-Cardiolipin at 0.1 : 0.9 peptide : lipid mole ratio (corresponding to 20 : 80 peptide : lipid covered area; curve (1) and at 0.22 : 0.78 peptide : lipid mole ratio (corresponding to 35 : 65 peptide : lipid covered area; curve (2). The subphase was NaCl 145 mM at pH 6. Arrow: collapse pressure corresponding to the peptide-enriched phase (immiscibility condition).

In order to see if the presence of a negatively charged lipid at the interface changes the behaviour of RTP when was mixed with the typical chloroplast lipids, MGDG and DGDG, POPG was added in the mixture at proportions near the biological composition reported for chloroplast membranes [26]. At any lipid to peptide proportions analysed, an immiscible behaviour was always observed (Figure 6(A) and (B)).

DISCUSSION

Cells have mechanisms that differentiate the signal sequences in the newly synthesized proteins that target and translocate secretory and membrane proteins to the endoplasmic reticulum membrane from those that are imported into chloroplasts. Also, the appropriate sequence distinguishes proteins imported into mitochondria from those that direct proteins to other subcellular organelles such as the nucleus or the peroxisome [2,27,28]. In chloroplast, the import of nuclear-encoded proteins is mediated by the interaction between the intrinsic N-terminal transit sequence of the preprotein and a common import machinery at the chloroplast envelope [1,15]. One of the general characteristic of signalling peptides that is emerging is the general amphiphilic pattern with similar properties rather than an exact sequence matching [6,7]. Thus, in the insertion or translocation into biomembranes the peptide-lipid interaction acquires relevance since it is taking part of the process. We have previously reported that 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 stability acquired for these peptides at air-water interface [19,29,30]. In contrast, the FT-IR spectra of the 24-residue chloroplast RTP either in aqueous solution or in presence of zwitterionic interface adopts essentially a

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

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)	1.7	—
(0.91/0.09)	2.65	Immiscible
(0.97/0.03)	2.83	—
RTP/DGDG	—	—
(0.77/0.23)	1.84	—
(0.91/0.09)	2.37	Immiscible
(0.97/0.03)	1.5	—
RTP/(MGDG-POPG 8 : 2)	—	—
(0.77/0.23)	2.3	—
(0.91/0.09)	2.21	Immiscible
(0.97/0.03)	2.73	—
RTP/(DGDG-POPG 8 : 2)	—	—
(0.77/0.23)	2.48	—
(0.91/0.09)	3.15	Immiscible
(0.97/0.03)	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 nm^2 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, the 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

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

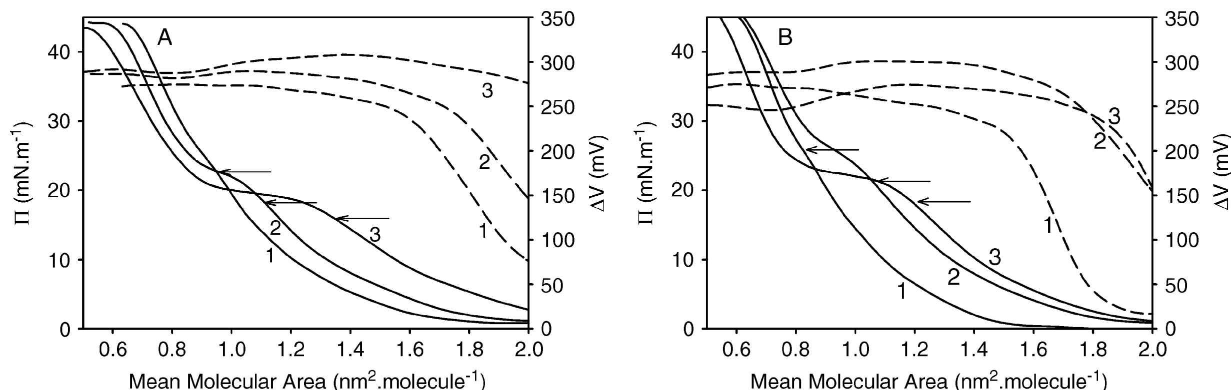

Figure 6 Π-Area compression isotherms of RTP/(chloroplast lipid/POPG 0.8 : 0.2 mole ratio) mixed monolayers. RTP/(MGDG/POPG, 0.8-0.2) (A) and RTP/(DGDG/POPG, 0.8-0.2) (B) at 0.03–0.97 peptide : lipid mole ratio (10 : 90 peptide : lipid covered area; curve (1), 0.1 : 0.9 (25 : 75 peptide : lipid covered area; curve (2) and 0.25 : 0.75 (50-50 peptide : lipid covered area; curve (3). Arrows: collapse pressure corresponding to the peptide-enriched phase (immiscibility condition).

behaviour is obtained the equivalent peptide molecular area changes substantially. This finding indicates that the peptide conformation or its interfacial location depends on both the type of lipid and its relative proportion at the mixed interface. The lower equivalent peptide area in the miscible region is compatible with either an α-helix parallel to the polar head groups of the lipids with some hydrophobic lateral residues protruding into hydrocarbon tails, or changes in the secondary structure with more beta-sheet content perpendicular to the interface, or a combination of both possibilities. Neither this behaviour obeys a particular physical state of the lipid, as it occurs with melittin peptide in which it differentiate between condensed and more liquid-expanded phospholipids [22], nor has RTP a general affinity for anionic interfaces. Also, in Figure 5 it can be observed that the mean molecular area of RTP/POPG monolayers at collapse pressure is lower when the peptide is immiscible than when it is miscible. On the other hand, this behaviour is not observed for cardiolipin. This fact can be interpreted as a loss of peptide/POPG from the monolayer when the peptide is at high proportions. This indicates how the lateral behaviour of mixed peptide/lipid monolayers depends on the proportion and quality of the components.

As a summary, here we report new data describing the behaviour of a chloroplast transit peptide when interacting with different interfaces (air–water, lipids, detergent interfaces). Here, we show that the surface properties and structure of the peptide depend on their quality. The biological relevance of monolayer studies is related to how RTP can be inserted inside the lipid bilayer and how it interacts laterally with the lipids at this stage when the protein is traslocated. As was described, RTP not only remains at the monolayer at high lateral pressures when anionic lipids are present at the peptide/lipid monolayer (*miscible behaviour*) but also interacts with anionic micelles adopting a more helical structure, presumably the one that is taken by RTP when it is incorporated into monolayers. Regarding the fact that RTP is not miscible with zwiterionic 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 is in the range of 20–35 mN m^{-1}; [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.

Acknowledgements

This work was supported by grants from CONICET, FONCYT (PICT 0609228), SECYT-UNC, and Agencia Córdoba Ciencia. G.D.F. is a member of the Career of Investigator. E.E.A. is a fellow from CONICET. The seminal experiments and peptide synthesis were made during a Welcome Trust fellowship support grant to G.D.F. at the Royal Free Hospital and St.George's Hospital, Medical School, London University.

REFERENCES

1. Chen X, Schnell DJ. Protein import into chloroplast. *Trends Cell Biol.* 1999; **9**: 222–227.
2. Agarraberes FA, Dice JF. Protein translocation across membranes. *Biochim. Biophys. Acta.* 2001; **1513**: 1–24.
3. Robinson C, Ellis RJ. Transport of proteins into chloroplasts. The precursor of small subunit of ribulose bisphosphate carboxylase is processed to the mature size in two steps. *Eur. J. Biochem.* 1984; **142**: 343–346.
4. Rusch SL, Kendall DA. Protein transport via amino-terminal targeting sequences: common themes in diverse systems. *Mol. Membr. Biol.* 1995; **12**: 295–307.
5. Smeekens S, Baurerie C, Hageman J, Keegstra K, Weisbeek P. The role of the transit peptide in the routing of precursors toward different chloroplast compartments. *Cell* 1986; **46**: 365–375.
6. von Heijne G. Transcending the impenetrable: how proteins come to terms with membranes. *Biochim. Biophys. Acta* 1988; **947**: 307–333.

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc

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 preferredoxin 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, Torok 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-bisphospate carboxulase. *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. Arrondo 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, Champman 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. *IUBMB 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; **41**: 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; **49**: 415–423.
37. Ambroggio EE, Kim DH, Separovic F, Barrow CJ, Barnham KJ, Bagatolli 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; **356**: 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.

Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

DOI: 10.1002/psc