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Effect of Increased Chain Packing on Gramicidin–Lipid Interactions†

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ABSTRACT: To study the effect of lipid packing on the dynamics of membrane proteins, the changes in the rotational motion of gramicidin tryptophans with increased packing brought about by high hydrostatic pressure through fluorescence spectroscopy were determined. In fluid phase dimyristoylphosphatidylcholine, the rotational motion of the residues decreased slightly with increased packing, but in the gel phase a significant reversible increase was observed. The magnitude of this increase was temperature dependent and much greater at lower temperatures. Quenching studies show that the increase in rotational motion is not due to a change in the location of the peptide in the membrane under pressure. Aromatic ring stacking between residues 9 and 15 appears to be stabilized under pressure, and there is no evidence of pressure-induced changes in peptide aggregation. The increase in rotational motion could be caused by a destabilization of hydrogen bonds between the indole hydrogens and the lipid head group oxygens due to an increase in the thickness of the compressible lipid bilayer with pressure without a concomitant lengthening of the peptide. These results indicate that specific interactions between lipids and proteins may play a major role of regulating the dynamics of membrane proteins.

The role the lipid matrix plays in controlling the structure and dynamics of integral membrane proteins is not fully understood. To a first approximation, membranes stabilize those conformations that will maximally allow for hydrophobic interactions with the lipid chains and more energetic interactions with the polar surface. These interactions are ultimately determined by the relative length of the lipid chain compared to the length of the hydrophobic surface of the protein. The dynamics or fast local motions of peptide residues should be related to the tightness of lipid chain packing. While several studies have been made on the effect of packing on membrane protein activity [e.g., Dornmair and Jahnig (1989) and Dannenberg et al. (1990)], which involves slower larger scale movements of the peptide backbone, little is known about the influence of packing on these smaller scale motions that are ultimately responsible for the larger conformational changes.

In this study, we will investigate the effect of membrane packing on the conformation and dynamics of gramicidin. Gramicidins are small peptides having the general formula (Sarges & Witkop, 1965)

HCO-LVal-Gly-LAla-DLeu-LAla-DVal-LVal-DVal-LTrp-DLeu-LX-DLeu-LTrp-DLeu-LTrp-NHCH$_2$CH$_2$OH

where X is Trp, Phe, or Try at a ratio of ~7:1:2 and where the NH_2-terminal Val is sometimes replaced by Ile. In membranes, these peptides have the ability to form channels selective for monovalent cations. The tertiary structure of gramicidin can vary from an intertwined helical dimer, as found in organic solvents, micelles, and certain membrane conditions to a formyl NH to formyl NH terminal dimer (referred to here as the N to N terminal dimer) of β 6.3 helices as seen in many bilayers [for a recent review, see Wallace (1990)].

The small size of the peptide lends itself well to determine the role each residue plays in ion permeation. One such approach has been to characterize the functional properties of gramicidin analogues with selected amino acid substitutions [e.g., Durkin et al. (1990)]. Recently, Becker and co-workers determined the functional properties of channels formed by GR[1] analogues having one to three Trp → Phe substitutions

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(Becker et al., 1991). When the number of Phe substitutions increased, a decrease in conduction as well as variations in duration was observed. These changes were thought to be related to the presence of hydrogen bonding between the indoles and the surface. The importance of the tryptophan residues in regulating the properties of the channel has also been noted by Jones et al. (1986), who found that photolysis of tryptophans lead to channel inactivation.

To better address the role tryptophan residues play in stabilizing the conformation of gramicidin in membranes, we will determine the effect of lipid packing on their rotational dynamics by fluorescence polarization. We have previously studied these motions as a function of temperature (Scarlata, 1988). In gel phase DMPC or fluid phase DOPC, no changes in tryptophan rotations, as seen by a constant polarization and lifetime (see eq 1), were observed through a large temperature range (5–25 °C and 5–40 °C, respectively). However, in fluid phase DMPC, the increase in rotational motion with temperature was larger than that expected from the thermal expansion of the bilayer. It was shown that this behavior was in part due to aromatic ring stacking between Trp 9 and 15, which holds these side chains tightly into place in gel phase DMPC but becomes disrupted at higher temperatures. In DOPC, data were indicative of a peptide aggregate stabilized by intermolecular stacking. However, since not all tryptophans participate in ring stacking in gel phase DMPC, this interaction alone cannot account for the complete insensitivity to temperature. In order to gain insight into the underlying cause of this behavior, we have conducted analogous studies using high-pressure fluorescence spectroscopy.

Pressure techniques have been extensively employed to study biological systems. In general, raising the pressure stabilizes aromatic ring stacking, dipolar interactions, and hydrogen bonding [for reviews, see Heremans (1982) and Weber and Drickamer (1983)]. We have conducted pressure studies of dansyl-gramicidin C (Teng et al., 1991). This peptide has a fluorescent dansyl residue attached to the tyrosine form of gramicidin. We found that the amount of energy transfer from dansyl to tryptophan, which is dependent on the distance between these residues, remained constant under pressure, indicating that the peptide conformation is relatively stable. It was also shown that the rotational motion of the dansyl residue decreases with increased lipid packing. In native gramicidin, the application of pressure should stabilize aromatic ring stacking and may allow us to determine the importance of ring stacking as opposed to other interactions that may aid in maintaining peptide conformation.

Materials and Methods

All lipids were from Avanti Polar Inc. (Birmingham, AL). Gramicidin and octyl glucoside were from Boehringer Mannheim Biochemicals (Indianapolis, IN) and dodecyl maltoside was from Calbiochem (San Diego, CA). Fatty acid probes were from Molecular Probes, Inc. (Junction City, OR). Gramicidin and fatty acid probes were incorporated into bilayers by cosonication as before (Scarlata, 1988).

Circular dichroism (CD) spectra were measured on a Jobin-Yvon Mark 5 spectrophotometer. Fluorescence data were taken on a Greg-PC (ISS, Champaign, IL) equipped with Glan-Thompson polarizers. The peptide was excited at either 280 or 303 nm and emission was collected at 345 nm. When scattered light accounted for more than ~2% of the signal, Corion interference filters were placed in the excitation and emission beams (303 and 350 nm, respectively). Phase-modulation fluorescence lifetimes under pressure were taken at the Laboratory for Fluorescence Dynamics at the University of Illinois with a Nd-YAG laser for a light source and excitation at 290 nm. Samples were subjected to pressure in a home-built chamber based on the design of Paladini and Weber (1981). Data were empirically corrected for pressure-induced window birefringence by a series of calibration curves composed of pressure data from *N*-acetyltryptophanamide and melittin monomers in methanol and buffer under identical optical conditions.

Depolarization through rotational motion is described by the Perrin equation:

$$A_0/A - 1 = (1/3 - 1/p)/(1/3 - 1/p_0) = RT\tau/\eta V \quad (1)$$

where A is the anisotropy, p the polarization, A_0 and p_0 are the limiting values observed in the absence of rotational motion, R is the gas constant, T is the absolute temperature, η is the viscosity, τ is the fluorescent lifetime, and V is the rotational volume when spherical motion is assumed. The value of A_0 of gramicidin with excitation at 303 nm, found to be approximately equal to its value at 305 nm, is 0.273 (Scarlata, 1988).

Results

Solvent and Micelle Studies. As a control, the effect of pressure on the rotational motion of gramicidin tryptophans was initially characterized in an isotropic solvent (butanol) and neutral micelles. Unless otherwise noted, fluorescence measurements were made by exciting the peptide on the red edge of the absorption spectrum (303 nm) to avoid tryptophan homotransfer and self-quenching (Weber, 1960). Butanol was chosen because of its ability to solubilize the peptide and its higher viscosity relative to the lower molecular weight solvents typically used. In organic solvents, gramicidin forms a mixture of right- and left-handed intertwined helices with the tryptophans residues protruding out into solution (Veatch et al., 1974). Consistent with this structure, a flat circular dichroism spectrum was obtained. In butanol at room temperature, the polarization of gramicidin was very low (0.110). This value represents the depolarization that results from tryptophan rotations, which are limited by both the local peptide environment and by any specific interactions between the indoles and the butanol (e.g., hydrogen bonding) along with the rotational motion of larger molecular volumes (up to 900 mL/mol from eq 1) that are experienced during the fluorescence lifetime of the tryptophans (~2.7 ns). Because of the low viscosity, we do not expect the polarization to be sensitive to the doubling of butanol viscosity induced in the 0.001–2 k bar pressure range (Bridgeman, 1958). However, if pressure disrupts specific interactions between the tryptophans and the solvent, then an increase in rotational motion would be observed. No changes in rotational motion as observed by the polarization and lifetime occurred in this pressure range, indicating that the butanol–gramicidin interactions probably remain intact.

Gramicidin was then studied in octyl glucoside micelles (55 mM). Again, the samples gave no apparent circular dichroism spectrum, which is consistent with an equilibrium combination of right- and left-handed intertwined helical dimers (Veatch et al., 1974). In order to isolate the changes in tryptophan

[1] Abbreviations: 2-, 6-, 9-, or 12-AS, 2-, 6-, 9-, or 12-(9-anthroyloxy)stearic acids; 11-AU, 11-(9-anthroyloxy)undecanoic acid; 16-AP, 16-(9-anthroyloxy)palmitic acid; dansyl, 5-(dimethylamino)-naphthalene-1-sulfonyl; DM, dodecyl maltoside; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; GR, gramicidin; OG, octyl glucoside; SUVs, small unilamellar vesicles.

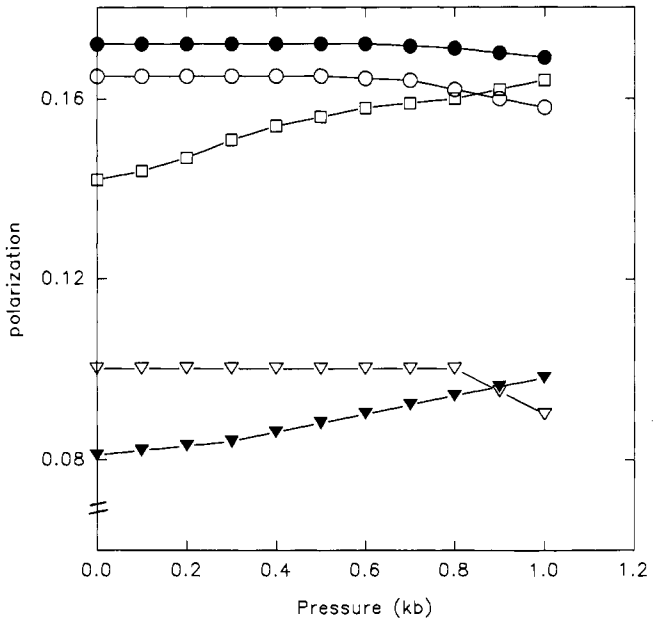

FIGURE 1: Polarization as a function of pressure for gramicidin in OG micelles in the absence (○) and presence (●) of 0.5 M KI and in DM (▽) micelles and 2-AS in OG (□) and DM (▼) micelles. Identical data were obtained for the 2-AS samples in the presence of 0.5 M KI.

rotational motion due to pressure-induced increases in micelle viscosity from changes due to local peptide interactions, we determined the pressure behavior of two fatty acid probes whose rotational behavior will reflect only the micelle environment. The probes chosen, 2-AS and 12-AS, have fluorophores that will detect fluidity changes at the surface (2-AS) and at more buried locations (12-AS). We note that since the peptide and the probe are in such low concentrations compared to the detergent, little or no quenching of the anthroyloxy groups by gramicidin was observed.

The polarization of 2-AS (Figure 1) and 12-AS (data not shown) displayed a large increase with pressure with no change in intensity. Under identical conditions, no significant changes in the polarization of gramicidin was observed (Figure 1) from 0.001 to 0.8 k bar (1 bar = 1.013 atm) where the micelle phase is stable. The fluorescence lifetime of gramicidin also remained fairly constant although the emission intensity increased, indicating a decrease in static quenching of the tryptophan residues. The change in intensity and lack of increase in polarization could be due to movement of the peptide from more surface-oriented, quenched, and rotationally restricted locations to more shielded, fluid, interior positions. We thus repeated the pressure experiment in excess of fluorescence quencher (0.5 M KI). The addition of KI resulted in a 31–34% loss of emission intensity compared to the same concentration of KCl with excitation both at the excitation maximum (280 nm) and at the red edge of the excitation band (303 nm). The presence of quencher caused the polarization to increase (0.173 as opposed to 0.165); however, no significant change in either the polarization or intensity was observed under pressure (Figure 1), indicating that the exposure of the tryptophans remained constant. Addition of KI did not effect the AS–OG results.

Although the lack of change in polarization could be due to the insensitivity of the relatively short-lived tryptophans residues to these lower viscosity changes, we note that in previous studies (Scarlata, 1988) the rotational motion of these residues in octyl glucoside micelles was very temperature sensitive from 5 to 40 °C. This decrease with increasing temperature was postulated to be due to a combination of a decrease in micelle viscosity and disruption of aromatic ring stacking. To better isolate these two effects, we repeated the pressure experiment at 40 °C, where the amount of stacking is reduced, and again no changes in polarization were observed.

The fact that we observed significant quenching in OG micelles indicated that a substantial population of the tryptophans are solvent exposed. Since the viscosity of water changes little in this pressure region (Bridgeman, 1958), it is possible that the tryptophans follow the viscosity of the aqueous solvent to a larger extent than the micelle. To test this hypothesis, we placed the peptide into micelles of the longer chain detergent dodecyl maltoside (0.5 mM). In these micelles, no quenching was observed upon the addition of 0.5 M KI. Again, the polarization and intensity remained constant with pressure even though a fatty acid probe, 2-AS, showed a smooth increase in polarization with no change in intensity (Figure 1). Thus, the solvent-shielded tryptophans are not sensitive to pressure-induced viscosity increases in micelles.

Gramicidin in Bilayers. We tested the sensitivity of the gramicidin tryptophans to pressure-induced fluidity changes for the peptide incorporated in DMPC small unilamellar vesicles (SUVs) at protein-to-lipid ratios between 1:200 and 1:50. The CD spectrum was consistent with the N to N terminal dimer conformation (Masotti et al., 1980; Wallace et al., 1981). Experiments were initially run at 28 °C, which is approximately 5 °C above the gel to fluid phase transition temperature. At this temperature, the onset of the gel phase transition occurs at ~0.4 kb (Melchoir & Morowitz, 1972; Heremans, 1982). Since our previous temperature studies showed no changes in rotational motion in gel phase in DMPC from 5 to 25 °C (Scarlata, 1988), we expected the polarization to either remain constant with pressure or increase due to stabilization of ring stacking. Instead, we found the polarization to reversibly decrease from 0.5 to 2 kbar (Figure 2A). When the temperature was lowered (7.5 °C) so that the sample is in the gel phase at atmospheric pressure, the polarization decreased sharply throughout the entire pressure range (Figure 2A).

One factor that may account for a decrease in polarization is an increased lifetime (see eq 1). However, we found that at room temperature the lifetime decreases a total of 430 ps from 0.001 to 2 kbars (Figure 3). We note also that there was an approximate 25% increase in fluorescence intensity with pressure indicating a loss of static quenching.

To determine the rotational behavior of the gramicidin tryptophans in the fluid phase, we repeated the experiments at 40 °C where the phase transition occurs at approximately 0.8 kb. A small increase in polarization was seen in the fluid phase, and a decrease was observed thereafter with a systematic increase in intensity (Figure 2A). In the fluid phase, we find that the decrease in rotational motion is approximately half that determined for a series of anthroyloxy fatty acid probes and dansyl-gramicidin C in DMPC SUVs (Scarlata, 1991; Teng et al., 1991). As will be discussed, this reduced sensitivity to changes in membrane packing could be attributed to changes in several interactions whose relative contributions cannot be isolated at this point.

Our data show that the decrease in polarization occurs only in gel phase DMPC. To determine whether the same is true for other lipids, we reconstituted the peptide in DOPC SUVs at a protein-to-lipid ratio of 1:50. The CD spectrum was consistent with an N to N terminal dimer. At room temperature (22 °C) these vesicles are in the fluid phase throughout the entire pressure range (Scarlata, 1991). Here,

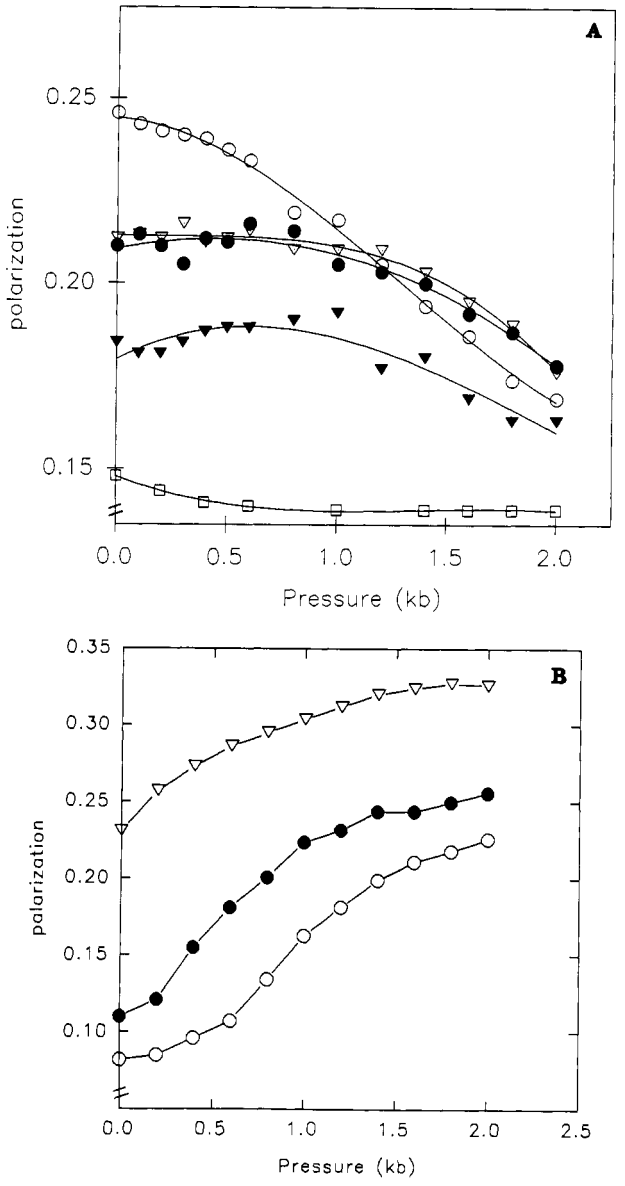

FIGURE 2: Polarization as a function of pressure for (A) gramicidin in DMPC at 28 °C in the absence (●) and presence (▽) of 0.5 M KI, at 7.5 °C (○) and 40 °C (▼) and in DOPC (□). (B) 11-AG-GR-DMPC at 7.5 °C (▽), 28 °C (●), and 40 °C (○).

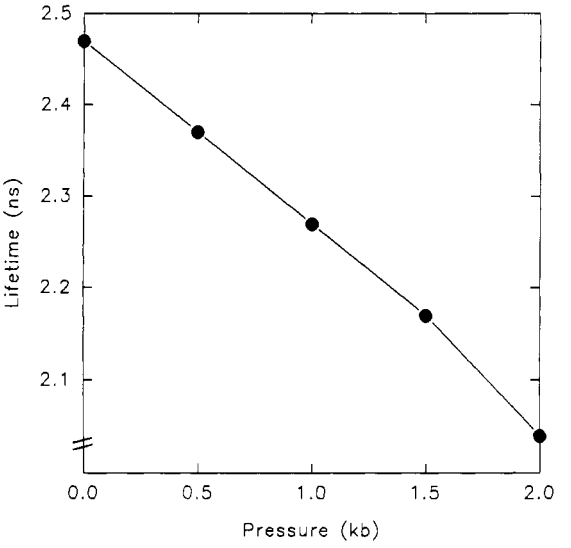

FIGURE 3: Fluorescence lifetime of GR-DMPC as a function of pressure at room temperature.

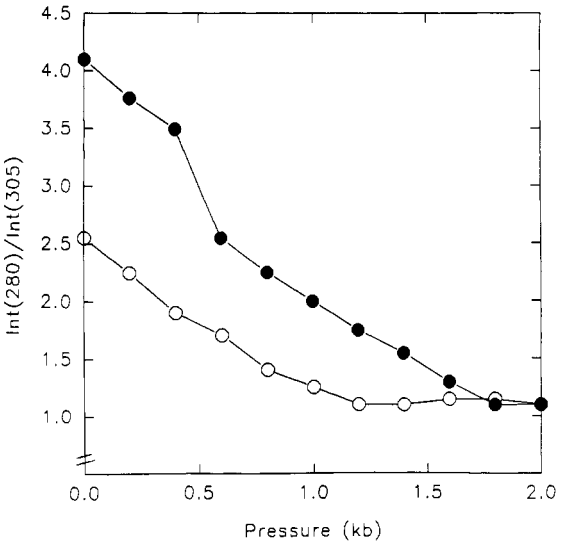

FIGURE 4: Ratio of the emission intensity of GR-DMPC with excitation at 280–305 nm as a function of pressure at (●) 40 °C and (○) 7.5 °C.

we find a small initial decrease in polarization with pressure that then remains constant (Figure 2A). Data were not taken in the gel phase since vesicle fusion occurred.

We note that the increase in intensity and decrease in polarization with pressure could be due to a decrease in either intramolecular or intermolecular ring stacking. The extent of ring stacking was monitored by the emission intensity ratio of the excitation wavelength where maximal self-quenching occurs (280 nm) to the wavelength where minimal self-quenching occurs (305 nm) (Scarlata, 1988). Because ring stacking is disrupted at higher temperatures, we monitored the 280/305 nm ratio at 7.5 and 40 °C. Accordingly, Figure 4 shows that, initially, the relative amount of self-quenching is much lower at the higher temperature. At both temperatures, self-quenching increases with pressure, indicating that stacking is stabilized in agreement with previously conclusions (Torgerson et al., 1980). Also, since intermolecular ring stacking has been shown to stabilize gramicidin aggregates (Killian et al., 1985), these data, along with other results (to be discussed), indicate that we are not changing the aggregation state of the gramicidin dimers under pressure.

Tryptophan has two major dipoles, which, because of their different orientations, have different polarization values and excitation spectra. In our experiments, we excited the peptide at 303 nm where the emission is solely from the L_{1b} band (Valeur & Weber, 1977). However, it is possible that pressure induces mixing of the two bands and the observed decrease in polarization is due to a greater percentage of the L_{1a} component. To determine whether this is the case, we recorded the excitation polarization spectra of the peptide in DMPC bilayers at 0.001, 1.0, and 2.0 kbar and observed an identical spectral shape from 290 to 305 nm (the correction for pressure-induced window birefringence became prohibitively large at lower wavelengths to obtain significant data). These results indicate that mixing is not occurring.

To ensure that the polarization–pressure behavior of gramicidin does not reflect unusual changes in the membrane itself, we conducted identical experiments on membranes labeled with the probe 11-anthroyloxyundecanoic acid (11-AU). This probe was chosen because its length is appropriate for myristoyl chains and the position of the probe would result in the least amount of quenching by gramicidin tryptophans (see below). The results for this series of experiments are shown in Figure 2B. We first compared the pressure behavior in the presence

and absence of gramicidin in DMPC (1:50 mol/mol) and found that the polarization increases smoothly and steeply with pressure as would be expected. Addition of KI has no effect on these values. Similar results were seen in DMPC SUVs labeled with 2-, 6-, 9-, and 12-AS probes (data not shown).

Changes in Peptide Location in the Membrane Under Pressure. It has been observed that some integral membrane proteins are eliminated from membranes under pressure (Muller & Shinitzky, 1981; Deckmann et al., 1985), which, if this occurred, would explain the decrease in polarization observed in Figure 2A. Therefore, we subjected GR–DMPC bilayers to pressure in the presence of quencher (0.08–0.5 M KI). The addition of KI resulted in an 8% decrease in intensity with no measurable change in the center of spectral mass, indicating that the tryptophans are well shielded from solvent quenchers. The pressure behavior of the polarization (Figure 3) and lifetime was identical with that seen in the absence of quencher, indicating that the tryptophans do not become more solvent exposed under pressure.

It is possible that the decrease in polarization with pressure is due to movement of the tryptophan residues into more fluid areas of the membrane. We investigated this possibility by monitoring the quenching of a series of anthroyloxystearic acid probes located at different membrane depths (2-, 6-, 9-, and 12-AS) by gramicidin tryptophans (Thulborn & Sawyer, 1978). In the N to N terminal dimer, the tryptophan residues lie close to the bilayer surface (Urry, 1971). Thus, we expect 2-AS to be the most quenched, followed by 6-, 9-, and then 12-AS. For DMPC SUVs in the gel phase at a probe/peptide/lipid ratio of 1:9:175, our results were comparable to those of Haigh et al. (1979) in that the normalized relative quenching efficiencies of 2-, 6-, 9-, 12-AS probes were 1.0, 0.8, 0.7, 0.55, respectively. In the fluid phase, the quenching pattern was marred by the fact that 2-AS gave variable signals. By monitoring the polarization response to the main phase transition, we found that in some samples 2-AS polarization was not always sensitive to the phase transition, indicating that the probe is not always embedded in the membrane. We also note that inaccuracies could be caused by variations in pH, the membrane concentration of the probe and/or incorrect position of the fatty acid probe in the membrane due to the shorter myristoyl chain of DMPC. Since the alignment of the tryptophans dipoles with respect to the anthroyloxy group on the AS probes may not be optimal for quenching, we also used 11-AU and 16-AP whose probe residues are at the end of the fatty acid chain rather than a side group. These probes showed far less quenching than their stearic acid counterparts in both DMPC and DOPC.

Even though the quenching pattern gave less than optimal results, we felt that we could use this method to measure the pressure dependence of quenching and determine whether the distance between the tryptophans and the particular probe changes with pressure. The pressure dependence of the intensities of GR–DPMC SUVs labeled with the series of anthroyloxy probes were measured and compared to labeled vesicles without peptide at 25 and 35 °C. Under pressure, we observed only small, unsystematic changes of less than 10% in probe quenching in either in the gel or fluid phases.

Discussion

In these studies we have found that the rotational freedom of gramicidin tryptophans increase with pressure in gel phase DMPC even though pressure causes a large increase lipid packing as seen by the decrease in the rotational motion of fatty acid probes. We have shown that the increase in tryptophan rotations is not due to the peptide being eliminated from the membrane or changing its membrane location. Nor is it due to changes in the excitation polarization spectrum, decreases in the amount of ring stacking, or a general consequence of lipid phase since a small increase in rotational motion was also observed in fluid phase DOPC. Although this behavior could be a result of conversion of the N to N terminal dimer to an intertwined helical dimer, we have several lines of evidence against this possibility: First, previous studies of dansyl-gramicidin C showed that the amount of energy transfer, which should be lower in the intertwined helical conformation, was constant with pressure (Teng et al., 1991). Second, the anthroyloxy fatty acid quenching pattern, which would be different for the intertwined helix where the tryptophans are more evenly distributed along the backbone (Haigh et al., 1979), remained constant with pressure. Third, the amount of ring stacking would be lower in the intertwined helical form, and we observe a stabilization of stacking under pressure. Although one may argue that increased ring stacking may be indicative of peptide aggregation, this mechanism is not consistent with a decreased polarization or the fatty acid quenching results. Nevertheless, even if conversion to the intertwined helix or another conformation did occur, we would still need to understand the underlying cause.

We have previously found that the change in rotational motion of dansyl-gramicidin C under pressure matched that of the anthroyloxy fatty acid probes in DMPC bilayers (Teng et al., 1991). We thus conclude that the increase in rotational motion is not due to unusual behavior of the peptide itself or the membrane. Since these studies indicated that the structure of the peptide was very compact in the bilayer, and no large-scale pressure-induced conformational changes were observed, then it is highly unlikely that the increased rotational motion we observe here for the unlabeled peptide is due to decreased interaction between the tryptophans and their neighboring amino acid residues.

On the basis of the above observations, we deduce that interactions between the tryptophans and the lipid or surface must change under pressure. The type of interaction that is most consistent with our results is hydrogen bonding between the indole hydrogens and either water or the lipid head groups. This conclusion is supported by the functional changes observed by Becker et al. (1991) for the Trp → Phe gramicidin analogues, along with gramicidin impermeability into phospholipid membranes (O'Connell et al., 1990), which was postulated to be due to the partially polar character of the indole groups. Recent Raman studies (Takeuchi et al., 1990) have reported that Trp 15, 13, and 11 interact with water. Since we only see an ~8% quenching residues by I^- ions, we conclude that these residues are in the hydrated head group region of the membrane but sufficiently shielded from aqueous phase ions. Hydrogen bonding between the tryptophan rings and the lipid head group or water is also consistent with the fact that the dansyl-labeled peptide and the anthroyloxy probes, which cannot donate a proton to hydrogen bond, show a systematic increase in polarization with pressure in gel phase DMPC (Teng et al., 1991; Scarlata, 1991). Functionally, hydrogen bonding may play a role in modulating the hydration of cations upon their entry and exit through the channel.

The combination of hydrogen bonding and ring stacking would severely limit the rotational motion of all the tryptophans and may result in the insensitivity of these motions to temperature changes in gel phase DMPC (Scarlata, 1988). In the more expanded fluid phase, we expect these interactions to be less energetic, resulting in an increased temperature sensitivity. Rupture of hydrogen bonds with temperature

would be consistent with the large increase in rotational motion with temperature previously observed in the intertwined helical form (gramicidin embedded in micelles) where one would expect less extensive ring interactions (Scarlata, 1988). Also, we note that hydrogen bonding would limit the change in polarization with pressure of the peptide in micelles.

Although these interactions explain the temperature data well, we note that hydrogen bonding occurs with a reduction of volume and should be stabilized under pressure. However, we (Scarlata, 1991) and others (Braganza & Worcester, 1986) have observed an increase in bilayer thickness with pressure due to straightening of the hydrocarbon chains. These latter investigators found the thickness of hydrated gel phase DPPC multilayers to increase by 1.5 Å at 19 °C in the 0.001–2 kbar range. This change, coupled with the ~30% increase in thickness bilayers undergo at the phase transition (Nagle & Wilkinson, 1978), would severely strain the specific peptide–lipid interactions. Peptides are almost incompressible compared to lipids (Weber & Drickamer, 1983). Because of this compressibility difference, pressure will increase the distance between the tryptophans and the lipid head group region, destabilizing hydrogens bonds. In the fluid phase, DMPC bilayers are expanded, and the rupture of hydrogen bonds is not extensive as seen by the increase in polarization with pressure. However, upon reaching the gel phase, the deformability of the bilayer can no longer accommodate the length mismatch. This argument than favors indole hydrogen bonding to the lipid head group oxygens rather than water since hydrogen bonding in this latter case would not be expected to be dependent on lipid chain length. The effect of chain length for this process is clearly seen by the DOPC results where the peptide is thought to be aggregated (Scarlata, 1988). The initial polarization decrease can be correlated to the relatively small amount of indole–lipid hydrogen bonding due to peptide aggregation (Scarlata, 1988), which reaches its final lower value after a few hundred bar. The tendency of the peptide to self-associate in longer chained lipids [see Killian et al. (1985)] precludes a systematic study of lipid length dependence.

Along with expanding the bilayer in the plane parallel to the hydrocarbon chains (**z**), pressure causes a large compression in the two perpendicular planes (Scarlata, 1991). Thus, pressure may also force the indole rings to become more **z** oriented, which may perturb the relative orientation of the molecules and destabilize hydrogen bonds. We note that the hydrogen bonds of water are disrupted in this pressure range, presumably for geometric reasons (Jonas et al., 1976; DeFries & Jonas, 1976). Movement of the indole rings to more **z**-oriented directions would also lessen the damping of tryptophan rotations by the hydrocarbon chains and work synergistically with hydrogen-bond rupture to lower the polarization. We do not feel that this latter effect alone would be completely responsible for the observed behavior because preservation of hydrogen bonds would still limit the polarization and not be consistent with previous temperature results.

Since pressure induces the dissociation of oligomeric proteins (Weber & Drickamer, 1983), it is possible that the gramicidin dimer also dissociates under pressure. However, dissociation of the dimer would not explain the decrease in polarization because the fluorescence lifetime is too short to be sensitive to the volume decrease that would occur upon dissociation whether or not spherical rotations are assumed (see eq 1). The monomers are held together by six intermolecular hydrogen bonds [see Wallace (1990)]. Since hydrogen bonds are formed with a reduction in volume, then, barring any specific geometric constraints, they should be stabilized by pressure. We thus expect the monomer–monomer hydrogen bonds to be stabilized at the expense of the peptide–lipid ones on the surface. However, if surface interactions remained intact, then a void could result in the middle of the membrane, which would disrupt chain packing and probably not be favored by pressure. Separation of the dimer may cause a differential behavior in the pressure–polarization plots in the presence and absence of peptide of a probe located in the region of the monomer–monomer interface (16-AP), and this is not seen. Rather than disrupting the center of the membrane, a deformation on the membrane surface would be more easily taken up by solvent, which would fill voids, preserve lipid chain packing, and replace any surface hydrogen bonds that may have broken.

These studies demonstrate that lipid packing can regulate the side-chain dynamics and conformation of membranes proteins by modulating specific protein–lipid interactions. By keeping the motions of the tryptophans in place, the dynamics of neighboring residues may also be damped, and the effect of packing in regards to a bulk "fluidity" effector may be secondary in some cases. Although gramicidin was chosen as a model system, the fact that it has several emitters that can either hydrogen bond, ring stack, or both makes it difficult to analyze the energies involved and their stabilizing effect on peptide conformation. We are presently repeating these studies using single tryptophans analogues that will allow us to isolate the relative contributions of these interactions. We would like to note that similar results have been obtained for bacterio-opsin (Scarlata, in preparation), where the majority of the tryptophans are also surface oriented. Combined, these studies point to the idea that membrane proteins specifically utilize tryptophans along with other polar residues to maintaining protein conformation through interactions with the membrane surface. The role these interactions play in peptide insertion into the membrane as well as the preference of a membrane protein for a particular lipid remains to be seen.

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In Vitro Interaction of a Polypeptide Homologous to Human Ro/SS-A Antigen (Calreticulin) with a Highly Conserved Amino Acid Sequence in the Cytoplasmic Domain of Integrin α Subunits†

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ABSTRACT: We endeavored to identify proteins interacting with KLGFFKR, a highly conserved motif in the cytoplasmic domain adjacent to the transmembrane domain of the α subunit of integrins. We found that affinity chromatography of cell extracts with this peptide followed by elution with EDTA resulted in the isolation of a 60-kDa protein (p60). The N-terminal amino acid sequence of this 60-kDa polypeptide was found to be highly homologous to the Ro/SS-A antigen, a 60-kDa protein homologous to calreticulin and Aplysia "memory molecule". The binding of p60 was found to be specific for the KLGFFKR sequence since this polypeptide did not bind to a peptide with a scrambled amino acid sequence (KLRFGFK), and it was also specifically eluted from the KLGFFKR affinity matrix ith soluble KLGFFKR peptide but not with the scrambled peptide. Solid phase in vitro binding assays demonstrated specific interaction of p60 with integrin α_3 and α_5 subunits but not with the β_1 subunit. Furthermore, p60 could be copurified with $\alpha_3\beta_1$ following coincubation in vitro. These interactions could be inhibited by KLGFFKR peptide and also by EDTA, indicating sequence-specific and divalent cation dependent binding. Despite the fact that calreticulin is thought to be localized in the endoplasmic reticulum, a pool of Ro/SS A antigen homologous 60-kDa polypeptide was found to be present in the soluble cytoplasm, indicating the feasibility of an interaction of p60 with the integrin α subunits. Our data suggest that p60 (Ro/SS-A Ag) can specifically bind to integrin α subunits via the highly conserved KLGFFKR amino acid sequence.

Integrins, a superfamily of cell surface receptors, provide a transmembrane link between the cell and the extracellular matrix (ECM) (Hynes, 1987; Ruoslahti & Pierschbacher, 1987). This transmembrane connection plays a central role in cellular adhesion, morphology, and migration. Included in this versatile family of integrins are the fibronectin, vitronectin, collagen, and laminin receptors. Some of the integrins interact with their respective ligands at a common tripeptide (Arg-Gly-Asp; RGD) recognition site. A typical integrin is a heterodimer consisting of an α and a β subunit in noncovalent association with one another. In some cases, the α subunit is composed of two disulfide-linked polypeptides that arise from proteolytic cleavage of a single precursor molecule. Amino

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