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Protective Effect of Lipidic Surfaces against Pressure-Induced Conformational Changes of Poly(L-lysine)†

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ABSTRACT: Poly(L-lysine) bound to phosphatidylglycerol or phosphatidic acid bilayers was submitted to hydrostatic pressure in a diamond anvil cell to investigate whether the lipidic surfaces can protect the polypeptide against pressure-induced conformational transformations. The amide I region of the infrared spectrum of dimyristoylphosphatidic acid bound polylysine shows that most of the polypeptide retains its β-sheet structure up to 19 kbar, while it is known to convert entirely to α-helix at $\sim$2 kbar in the absence of the lipid [Carrier, D., Mantsch, H. H., & Wong, P. T. T. (1989) *Biopolymers* (in press)]. The simultaneous binding of the polypeptidic molecules to two opposing bilayers appears to be required in order to preserve the β-sheet structure at pressures over $\sim$9 kbar: a small proportion of the polypeptide, most likely the molecules at the surface of the aggregated bilayers, was found to convert to unordered and eventually to α-helical conformations in the pressure range 9–19 kbar. The decrease from 1612 to 1606 cm^{-1} of the frequency of the major β-sheet component of the infrared amide I band as the pressure is raised to 6 kbar indicates a strengthening of the interchain hydrogen bonds. The high-pressure infrared spectra of polylysine bound to dimyristoyl- and dipalmitoylphosphatidylglycerol show that the polypeptide remains α-helical up to $\sim$12 kbar, though the changes in the bandshape indicate an increase in hydrogen bond strength. The formation of a small amount of β-sheet was observed during decompression and is attributed to the effect of dehydration on the polypeptidic molecules located at the surface of the aggregates. This study suggests that lipidic surfaces could be used as cocatalysts, to control and protect the active conformation of enzymatic proteins for operation at elevated hydrostatic pressure.

Poly(L-lysine) is frequently used as a model protein because of its ability to adopt three different conformations: α-helix, β-sheet, and an unordered structure which has been proposed to actually consist of extended helices (Krimm & Tiffany, 1974; Paterlini et al., 1986). A high pH value (>10.5) is required for the formation of the first two conformers in aqueous solution; nearly pure α-helix or β-sheet conformations can be obtained if the temperature is maintained below 4 °C or raised above 50 °C, respectively. The conformation of polylysine can also be modulated by other means. At neutral pH, it forms α-helices upon binding to phosphatidylglycerol bilayers (Carrier & Pézolet, 1984) while binding to phosphatidic acid bilayers is accompanied by conversion to β-sheets (Laroche et al., 1988). More recently, hydrostatic pressure has been shown to promote the formation of α-helices from unordered as well as from β-sheet polylysine in aqueous solution (Carrier et al., 1989). The purpose of the present infrared spectroscopic study is to investigate the effect of high pressure on the polypeptide already bound to dimyristoylphosphatidic acid (DMPA),[1] DPPG, or DMPG. In other words, we want to establish whether the surface of a lipidic bilayer can protect the polypeptide against pressure-induced denaturation.

Experimental Procedures

Poly(L-lysine) hydrobromide of molecular weight 271 000 and the sodium salt of dimyristoyl-L-α-phosphatidic acid

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[1] Abbreviations: DMPA, dimyristoylphosphatidic acid; DMPG, dimyristoylphosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; FT-IR, Fourier-transform infrared.

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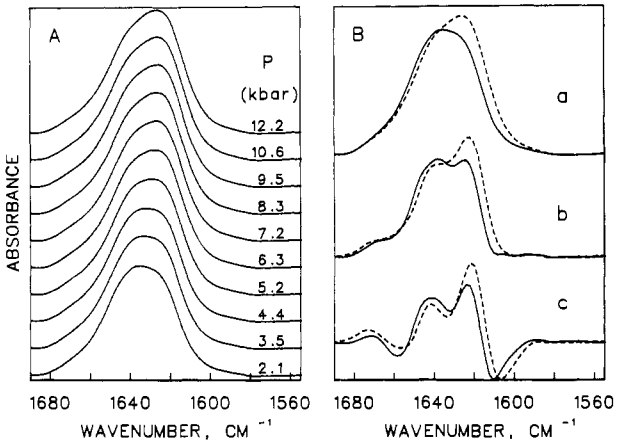

FIGURE 1: (A) Amide I region of the infrared spectra of equimolar DPPG/polylysine prepared in 50 mM phosphate buffer/^2H_2O, p^{2}H 7.5. Spectra were recorded at increasing pressure. (B) Superimposed infrared spectra corresponding to the lowest (solid traces) and highest (dashed traces) pressures before (a) and after band narrowing by Fourier self-deconvolution using a bandwidth of 15 and a resolution enhancement factor of 2.2 (b) or by Fourier derivation using a power of 2 and a breakpoint of 0.5 (c).

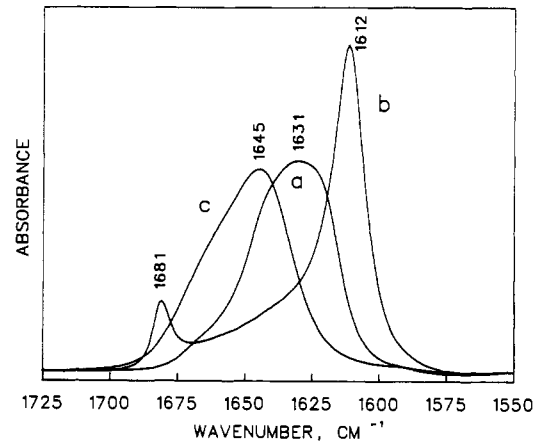

FIGURE 2: Amide I region of the infrared spectra of a 2 wt % solution of poly(L-lysine) in 0.2 M NaCl/^2H_2O at p^{2}H 11.1 and 5 °C (a) or 34 °C (b). Spectrum c corresponds to a 2.5 wt % solution of poly(L-lysine) in deuterated water at 25 °C and neutral p^{2}H.

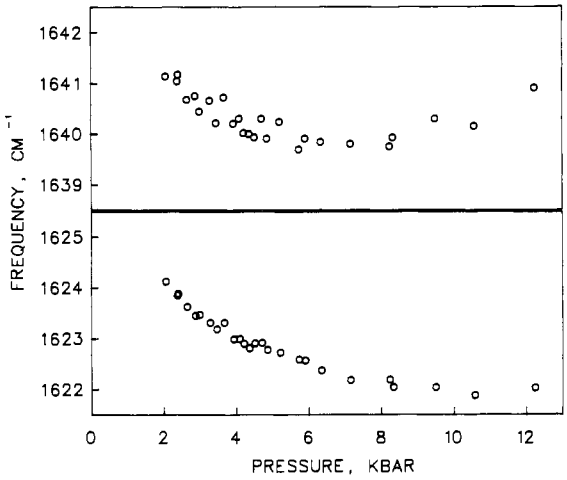

FIGURE 3: Pressure dependence of the frequency of the components of the amide I band of DPPG-bound polylysine. The frequencies were obtained from spectra after Fourier derivation with a power of 2 and a breakpoint of 0.4 (see Figure 1 for other details).

(DMPA) were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium salts of dimyristoyl- and dipalmitoyl-L-α-phosphatidylglycerol (DMPG and DPPG) were from Avanti Polar Lipids, Inc. (Birmingham, AL), and deuterated water was from MSD isotopes (Montréal, Canada). Lipidic dispersions (0.5 wt %) were prepared in 50 mM phosphate/^2H_2O (p^{2}H 7.5) buffer, with at least three freeze–thaw cycles. The required amount of 0.5 wt % solution of polylysine in the same buffer was then added to each lipidic dispersion and the mixture submitted to at least three additional freeze–thaw cycles before centrifugation. The white pellet was used for infrared measurements.

The samples were placed in a 0.37-mm-diameter hole on a 0.23-mm-thick stainless-steel gasket together with a small amount of powdered α-quartz and mounted on a diamond anvil cell. Pressures at the sample were determined from the 695 cm^{-1} infrared absorption band of α-quartz, as described earlier (Wong et al., 1985).

Infrared spectra at various pressures were measured on a Digilab FTS-60 Fourier-transform spectrometer using a liquid nitrogen cooled mercury–cadmium–telluride detector. A total of 512 interferograms were co-added for each spectrum, with a spectral resolution of 4 cm^{-1}. Data treatment was performed as described earlier (Mantsch et al., 1988).

To obtain spectra of a basic aqueous solution at atmospheric pressure, polylysine was dissolved in cooled 0.2 M NaCl/^2H_2O and the p^{2}H adjusted with NaO^{2}H. The sample was quickly transferred onto precooled CaF_2 windows and assembled into a cold Harrick cell using a 56-μm Teflon spacer. The sample at neutral pH was prepared by dissolving the polylysine hydrobromide in ^2H_2O. The temperature was regulated by a circulating fluid from a thermostated bath. The spectra were measured with a Digilab FTS-60 Fourier-transform spectrometer using a high-sensitivity TDGS detector. A total of 256 scans were collected with a resolution of 2 cm^{-1}. For each sample, the spectrum of the corresponding solvent was measured under identical conditions and digitally subtracted.

Results

As was shown by Raman spectroscopy (Carrier & Pézolet, 1984), poly(L-lysine) adopts an α-helical structure when it binds to DPPG bilayers. This is confirmed by the infrared spectra (Figure 1) of polylysine bound to a multilamellar dispersion of DPPG, at a molar ratio of one lipid molecule per lysine residue. The amide I band of the infrared spectrum measured at the lowest pressure investigated, i.e., 2.1 kbar (bottom trace of Figure 1A), bears a close resemblance to the spectrum of the α-helical conformer of free polylysine (that is in the absence of the lipid) in solution at atmospheric pressure (Figure 2a), though the relative intensities of the components of the two envelopes differ slightly.

As the pressure is increased from 2.1 to 12.2 kbar (Figure 1A), the shape of the amide I band of DPPG-bound polylysine changes gradually, and the band maximum shifts from 1635 to 1626 cm^{-1}. A similar bandshape modification is observed when aqueous solutions of polylysine are compressed above the pressure at which the polypeptide converts from β-sheet or unordered structures to α-helices (Carrier et al., 1989). Spectral resolution enhancement by Fourier self-deconvolution (Figure 1Bb) or Fourier derivation (Figure 1Bc) reveals the presence of two major components, at 1624 and 1642 cm^{-1}, in the 2.1-kbar spectrum of DPPG-bound polylysine (solid trace). At 12.2 kbar (Figure 1B, dashed trace), the low-frequency component has increased in intensity and shifted to 1622 cm^{-1}. The variation of the frequency of each component as a function of pressure is shown in Figure 3. Below ~6 kbar, both components decrease in frequency, reflecting a pressure-induced strengthening of the hydrogen bonds between

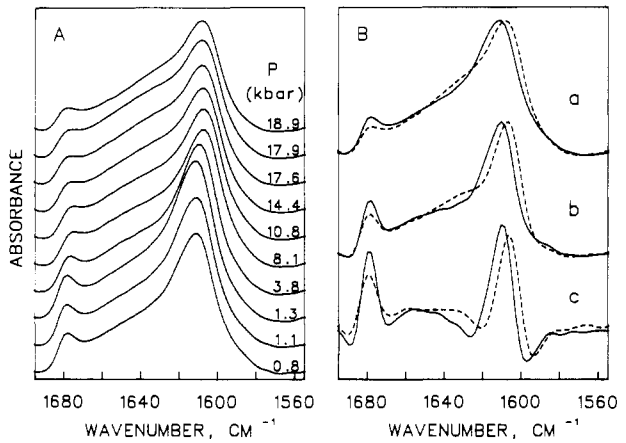

FIGURE 4: (A) Amide I region of the infrared spectra of equimolar DMPA/polylysine prepared in 50 mM phosphate buffer/^2H_2O, p^{2}H 7.5. Spectra were recorded at increasing pressure. The sample was kept under a pressure of 18.9 kbar for a period of 18 h before the top spectrum was recorded. (B) Superimposed infrared spectra corresponding to 1.1 kbar (solid line) and 18.9 kbar (dashed line) before (a) and after band narrowing by Fourier self-deconvolution using a bandwidth of 12 and a resolution enhancement factor of 1.5 (b) or by Fourier derivation using a power of 2 and a breakpoint of 0.5 (c).

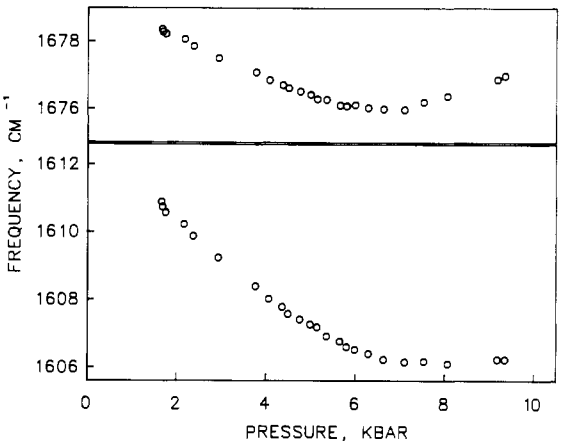

FIGURE 5: Pressure dependence of the frequency of the components of the amide I band of DMPA-bound polylysine. The frequencies were obtained from spectra after Fourier derivation with a power of 2 and a breakpoint of 0.4 (see Figure 4 for other details).

amide groups (Wong, 1987). Above ~6 kbar, the slope becomes either very small (low-frequency component) or positive (high-frequency component), indicating that a second, opposing effect has become more important. The shift to higher frequency is due to the amide bond compression resulting from the increased intermolecular repulsion force at elevated pressure (Wong, 1987). The DMPG-bound polylysine exhibited a similar behavior, except that below 6 kbar the frequencies of the amide I components were slightly (1–2 cm^{-1}) lower.

The spectrum of DMPA-bound polylysine at 0.8 kbar (Figure 4A) exhibits the distinctive features of β-sheet: an intense, narrow band at 1611 cm^{-1} and a weak peak at 1678 cm^{-1}. These bands compare well with those obtained for the β conformer of free polylysine in aqueous solution at atmospheric pressure (Figure 2b). For the low-frequency component of the β-sheet conformer, the decrease in frequency between 0 and 6 kbar is roughly twice that found for the α-helical structure (compare Figures 5 and 3). This is not surprising since strengthening of the hydrogen bonds requires a compression of the helix and therefore changes in bond angles for the α conformer, whereas in the β-sheet polylysine, the peptidic chains simply have to be brought closer. Yet, the frequency of the $\nu(\pi,0)$ mode is already very low at normal pressure (1612 cm^{-1}), compared to the more common value of ~1635 cm^{-1} found for the β-sheets of globular proteins. The exceptionally small value observed for DMPA-bound polylysine at high pressure, i.e., 1606 cm^{-1} (Figure 5), indicates extremely strong hydrogen bonds. Therefore, in the pressure range investigated, the nature of the polylysine side chains favors a geometry of the polypeptide backbone that is especially well suited for hydrogen bonding between amide groups.

Free polylysine in 1.5 wt % aqueous solution converts from β-sheet to α-helix at ~2 kbar (Carrier et al., 1989). However, no indication of such a β-sheet to α-helix conversion could be observed with DMPA-bound polylysine up to about 9 kbar (Figure 4). Both amide I peaks are slightly broadened and shift to lower frequencies because of the pressure-induced distortion and increase in hydrogen bond strength, respectively. Above ~8 kbar, there is a small increase in intensity in the area between 1630 and 1660 cm^{-1}. This is attributed to a minor increase in unordered conformers above that pressure and could indicate a pressure-induced dissociation of the polypeptide, a simple scrambling of the geometry, or a transient state during conversion to α-helix. In order to address this question, the sample was maintained at the highest pressure (18.9 kbar) for 18 h. In the spectrum obtained after this waiting period (Figure 4B, dashed line), the intensity between approximately 1645 and 1670 cm^{-1} has decreased to its initial level below 8 kbar (relative to the major β-sheet peak), whereas a concomitant increase in intensity was observed at ~1633 cm^{-1}. This indicates that the β-sheet polypeptidic molecules that had become unordered have then slowly converted to α-helices. Despite the long incubation time allowed at 18.9 kbar, the β-sheet conversion did not progress further and seems to be only a marginal process. It is likely that it affects only the molecules bound to a single bilayer, which are "half-stabilized" compared to those involved in bridging successive bilayers. High pressure generally promotes dissociation of ionic species because the solvent water molecules pack closer around charged solute molecules (Neuman et al., 1973). The dissociated polypeptidic molecules then convert to the α-helix conformation, in agreement with earlier experiments on free polylysine in aqueous solutions (Carrier et al., 1989).

The pressure-induced changes in the infrared spectra of DMPA-bound polylysine were found to be completely reversible upon pressure release (not shown). However, new components appeared in the amide I band of DPPG- and DMPG-bound polylysine as the samples were decompressed. The solid line in Figure 6A shows the spectrum of DMPG-bound polylysine measured at 1.4 kbar during compression, while the dashed line shows the spectrum obtained at the same pressure after decompression. The two positive peaks at 1678 and 1614 cm^{-1}, along with the negative signal at 1634 cm^{-1} in the infrared difference spectrum (Figure 6B), show that some of the α-helices have been converted into β-sheets.

Discussion

The frequency (1631 cm^{-1}) and half-width (36 cm^{-1}) of the infrared amide I band of α-helical poly(L-lysine) in Figure 2a agree well with the results of Chirgadze and Brazhnikov (1974) obtained under similar conditions (1633 and 38 cm^{-1}). From optical rotatory dispersion, these authors estimated the helical content of their sample to be about 85%. A weak band at ~1665 cm^{-1}, appearing as a shoulder in the spectrum in Figure 2a, is always present in polylysine because the latter forms rather short helices (Gill et al., 1972; Applequist & Doty,

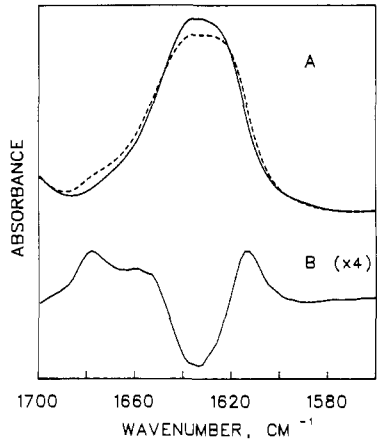

FIGURE 6: (A) Amide I region of the infrared spectra of equimolar DMPG/polylysine, prepared in 50 mM phosphate buffer/^2H_2O, p^{2}H 7.5. Spectra were recorded at 1.4 kbar during compression (solid line) and following decompression (dashed line). (B) Infrared difference spectrum obtained by subtraction of the spectrum recorded upon compression from that recorded during decompression.

1962). While the amide I band of α-helical polypeptides usually appears as a broad, symmetrical band, a substructure is observed in our spectra of α-helical polylysine, both free in solution (Figure 2a) and when bound to DPPG bilayers (Figure 1).

An analysis of the vibrational spectra of α-helical polypeptides using symmetry considerations leads to the conclusion that the infrared amide I band consists of two components, a strong ν_A and a weak ν_E component, which are 2–4 cm^{-1} apart and therefore unresolvable due to the broadness of the experimental spectra (Chirgadze & Brazhnikov, 1974; Krimm & Bandekar, 1986). It is tempting to speculate that the two major components seen in Figure 1B would correspond to these two different modes, the frequency difference in poly(L-lysine) being for some reason larger than usual. In early experiments with films of poly(L-lysine) deuteroiodide in α-helical conformation (Elliott et al., 1975), a strong infrared band was observed at ~1635 cm^{-1} when the plane of the polarized radiation was oriented parallel to the fiber axis, and a medium band with perpendicular dichroism was observed at ~1645 cm^{-1}. The increase in intensity of the low-frequency ν_A component under high pressure (Figure 1) could then be related to an improved alignment of the carbonyl groups along the compressed helix, leading at the same time to stronger hydrogen bonds, as reflected by the observed decrease in frequency (Figure 3). However, a few factors argue against such an interpretation. First, the intensity of the ν_A mode should be much stronger than that of the ν_E modes. Miyazawa and Blout (1961) give a 5:1 ratio for the ν_A/ν_E intensities. On the other hand, the ν_A mode is also expected to be very intense in the Raman spectrum of α-helical polypeptides, but Krimm and Bandekar (1986) reported that only the ν_{E_1} vibration could be observed in α-helical poly(L-alanine). Second, the above interpretation of the substructure of our infrared spectra of α-helical polylysine implies a very large frequency difference between the two components, that is, approximately 18 cm^{-1} compared to the predicted splitting of 2–4 cm^{-1}. The spectra of α-helical polypeptides are usually consistent with a small ν_A–ν_E splitting, a high ν_A:ν_E intensity ratio, and the degeneracy of the two E modes, but it should be emphasized that these characteristics were obtained from the analysis of infinite helices. As mentioned above, poly(L-lysine) forms short helices. The infrared spectra of polypeptidic helical fragments containing 2–18 residues have been calculated by using the perturbation theory in a dipole–dipole approximation (Nevskaya & Chirgadze, 1976a). In these spectra, the two ν_E modes appear at distinct frequencies, the ν_A mode is not always the most intense, and E-type vibrations of a higher order become active. The intensities, the frequencies, and the splittings change dramatically with the number of residues, giving the calculated spectra very different shapes. Although none of them corresponds exactly to our experimental spectra, they demonstrate that the assignment proposed above is reasonable. It should be emphasized that there are several inherent assumptions in normal-mode analyses, which generally deal only with idealized, isolated systems. In particular, they cannot account for the various influences experienced by polypeptidic molecules in the condensed phase, e.g., solvent and salt concentrations. As pointed out by Chirgadze and Brazhnikov (1974), the half-width of the amide I band of polypeptidic α-helices can vary between 15 and 40 cm^{-1}, depending on the conditions. Water broadens the amide I band due to destabilization of the helix. A variety of conformers is created, and their respective amide I signals add up to produce the global envelope that is measured experimentally. In addition to differing geometries of the amide groups, the bandshape is also modulated by differences in the length, orientation, and phase angle of the individual helices. From such a perspective, the changes observed in this study (Figure 1) indicate that helices with stronger hydrogen bonds are favored at high pressure. The hydrostatic pressure can affect both the backbone geometry and the pitch of the helix. As the hydrogen bonds between neighboring turns strengthen, the carbonyl bonds become weaker and more parallel to the long axis of the helix. From the theory of resonance interactions of transition dipoles, the resulting changes in the magnitude and direction of the transition dipole moment are expected to lead to an increase in intensity of the A-type band and to a decrease of the E modes, as observed in the present study (Figure 1).

The spectral changes observed for β-sheet under high pressure (Figures 4 and 5) have also been interpreted in terms of hydrogen bond strengthening and geometry distortion. From the theory of transition dipole–transition dipole interactions, it has also been shown that the frequency of the $\nu(\pi,0)$ mode decreases as the number of polypeptidic chains per β-sheet increases and reaches a plateau at about seven chains (Nevskaya & Chirgadze, 1976b). The numer of chains per sheet is certainly a relevant parameter in solution, but we believe that its effect is negligible for polylysine bound to DMPA bilayers. At the beginning of the pressure run, the frequency of the major band of β-sheet polylysine bound to DMPA is already lower than that of the free β-sheet polypeptide, which forms such extensive sheets that they are visible to the naked eye. This indicates that the sheets formed by DMPA-bound polylysine contain a large number of chains. The presence of the bilayer surfaces favors an efficient alignment of the chains to produce large, monomolecular sheets sandwiched between the stacked bilayers in the resulting aggregates.

Our experiments with DMPA show that a lipidic surface can in fact be used to prevent pressure-induced conformational changes in polylysine. While β-sheet polylysine in aqueous solution has been shown to convert to α-helix at ~2 kbar, the DMPA-bound β-sheet polylysine retained this conformation up to ~9 kbar. However, in the pressure range between 9 and 19 kbar, a minor portion of the polypeptide—most likely the molecules facing the bulk solvent—became unordered and then α-helical. Even so, the vast majority of polylysine molecules kept their initial β-sheet conformation.

Many membrane proteins are known to become inactive after delipidation (Sandermann, 1978; Martonosi, 1963). While these proteins are usually intrinsic and therefore hydrophobic, the high hydrophilicity of polylysine makes it an ideal model for extrinsic proteins. Nevertheless, the behavior of this polypeptide under pressure has been shown here to be modified by its binding to lipidic surfaces. The dominance of surface effects over pressure effects indicates that lipid bilayers are potential surface catalysts for protein reactions: one could imagine systems in which the formation of the active structure of a protein would be triggered by the proper lipidic surface and maintained against pressure to carry on a specific reaction having an enhanced yield at elevated pressure.

Since phosphatidylglycerol headgroups and high pressure both favor the α-helical conformation of poly(L-lysine), no major conformational change was expected for DMPG- and DPPG-bound polylysine. The appearance of some β-sheet during decompression (Figure 6) is thus surprising, but it was observed in all experiments. Since it occurred only during pressure release and only a minor portion of the polypeptide was affected, this conversion is most likely due to dehydration: no sample loss can occur during compression, but decompression may be accompanied by leakage and solvent evaporation in the nitrogen-purged compartment of the FT-IR instrument. Actually, the intensity of the HO^2H band at $\sim$3300 cm^{-1} was observed to decrease upon decompression in all experiments, supporting such a contention. Poly(L-lysine) films are known to convert successively from β-sheet to α-helix to random coil upon hydration (Shmueli & Traub, 1965; Blout & Lenormant, 1957; Elliott et al., 1957; Suwalsky & Llanos, 1977). On the other hand, polylysine–DPPG complexes have been shown to consist of stacked DPPG bilayers, separated by monolayers of α-helical polylysine (Carrier & Pézolet, 1986). The formation of these sandwich structures produces a visible aggregation. Water is expelled from the interbilayer space, but some solvent can remain between the aggregates. Therefore, the polylysine helices bound to the surface of the aggregates might be affected by dehyration and thus transform to β-sheet. Most poylysine molecules, however, are bound to opposing bilayers and should not sense the interstitial dehydration and therefore not convert to β-sheet.

In conclusion, external hydrostatic pressure up to 12 kbar failed to dissociate or change the conformation of both β-sheet polylysine bound to DMPA and α-helical polylysine bound to DMPG or DPPG. The amide I region of the infrared spectra of the two conformers revealed an increase in the strength of the hydrogen bonds between the backbone amide groups as the pressure was raised to 6 kbar. For phosphatidylglycerol-bound polylysine, the ν_A component increased in intensity at high pressure due to the distortion of the α-helices. The DMPA surface was shown to provide effective protection against the pressure-induced change in conformation from β-sheet to α-helix up to $\sim$9 kbar for molecules bound to a single DMPA bilayer and up to at least 19 kbar for molecules linked to opposing surfaces.

Registry No. DMPA, 30170-00-4; DMPG, 61361-72-6; DPPG, 4537-77-3; poly(L-lysine), 25104-18-1; poly(L-lysine) (SRU), 38000-06-5.

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