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A Study on the C-Terminal Membrane Anchoring of *Escherichia coli* Penicillin-Binding Protein 5

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***Escherichia coli* penicillin-binding protein 5 (PBP5) anchors to the inner membrane in a pH-dependent manner via a C-terminal amphiphilic α-helix. Low pH was found to enhance both levels of PBP5 membrane anchoring and levels of α-helicity in an aqueous PBP5 C-terminal homologue, which led to the suggestion that levels of PBP5 membrane anchoring are related to levels of PBP5 C-terminal α-helicity. Here we have used Fourier-transformed infrared spectroscopy (FTIR) and a peptide homologue of the PBP5 C-terminal sequence to investigate the effect of pH on the conformational behavior of this sequence at a lipid interface and on its ability to interact with lipid. Our results suggest that the membrane-anchoring mechanism of PBP5 is unlikely to involve conformational change in the protein's C-terminal region and may therefore involve conformational changes in the protein's ectomembranous domain.**

***Key Words: E. coli* PBP5; C-terminal α-helix; conformational change; membrane.**

Escherichia coli low molecular mass penicillin-binding protein PBP5 is believed to play a regulatory role in cell wall assembly and is anchored to the periplasmic face of the inner membrane (1, 2). The membrane anchoring of PBP5 exhibits a pH dependent susceptibility to urea extraction, for at low pH, PBP5 is resistant to the perturbant but becomes increasingly susceptible as pH is increased above neutrality (3, 4). More recent studies have shown that the urea-resistant and urea-susceptible forms of PBP5 are legitimately membrane bound and exist in a state of conformational equilibrium (4).

Based on the results of a number of theoretical analyses (5–7) and experimental studies (8–10), it has been suggested that PBP5 may anchor to the membrane via a C-terminal amphiphilic α-helix. Supporting this suggestion, a peptide homologue of the PBP5 C-terminal region (Table 1) was found to penetrate model membranes (11) and to adopt α-helical structure in aqueous solution (12) with a pH dependence that correlated to that of PBP5's susceptibility to urea. These results led to the suggestion that the urea resistant and susceptible forms of PBP5 may differ in the levels of their membrane anchoring and that interconversion between these forms may involve pH related conformational changes within the protein's C-terminal region (1, 13). Nonetheless, it was found that, when the PBP5 anchor region was fused to a soluble periplasmic β-lactamase, although the hybrid protein was able to anchor to the membrane, it was unable to adopt the urea resistant state (14). Furthermore, using an active-site mutant, these latter workers (14) found that when PBP5 interacted with a β-lactam antibiotic, and was therefore in an active conformation, the ability of the protein to adopt the urea resistant state was greatly reduced. Based on these observations, it was proposed that, although the presence of the PBP5 C-terminal region appeared necessary, it was not sufficient for membrane anchoring of the protein (14). Conformational changes within the PBP5 ectomembranous domain may also be a determinant of the level of membrane interaction shown by the protein and be responsible for the interconversion of the urea resistant and susceptible forms of PBP5.

Here we have used Fourier-transformed infrared spectroscopy (FTIR) and P5, a peptide homologue of the PBP5 C-terminal sequence (Table 1) (15), to investigate the conformational behavior of this sequence at a lipid interface and its ability to interact with lipid.

MATERIALS AND METHODS

Materials. P5 (Table 1) was supplied by PEPSYN (Liverpool, UK), synthesized by solid-state synthesis, purified by HPLC, and showed a purity of 99% or greater. The phospholipids dimyristoyl phosphatidylglycerol (DMPG), dimyristoyl phosphatidylcholine (DMPC), and dimyristoyl ethanolamine (DMPE) and all solvents were purchased from Sigma.

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TABLE 1

The Primary Structure of the PBP5 C-Terminal Region

Protein	C-terminal residues
	+ − + ++ −
PBP5	GNFFGKIIDYIKLMFHHWFG-COO

Preparation of phospholipid small unilamellar vesicles. Small unilamellar vesicles (SUVs) were prepared according to the method of Keller *et al.* (16). Essentially, lipid/chloroform mixtures were dried with nitrogen gas and hydrated with aqueous buffer {50 mM Hepes (*N*-[2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid]), pH 7} to give a final phospholipid concentration of 5 mM. The resulting cloudy suspensions were sonicated at 4°C with a Soniprep 150 (amplitude 10 μm) until clear suspensions resulted (30 cycles of 30 s) which were then centrifuged (15 min, 3000*g*, 4°C).

FTIR conformational analyses of P5. To give a final peptide concentration of 1 mM, P5 was solubilized in suspensions of SUVs, which were formed from DMPG, DMPC, or DMPE and were prepared as described above. Samples of solubilized peptide were spread on a CaF_2 crystal, and the free excess water was evaporated at room temperature. The single-band components of the P4 or P5 amide I vibrational band (predominantly C═O stretch) was monitored using an FTIR "5-DX" spectrometer (Nicolet Instruments, Madison, WI) and for each sample, absorbance spectra produced.

Analysis of FTIR spectra. FTIR spectra were analyzed and for those with strong absorption bands, the evaluation of the band parameters (peak position, band width, and intensity) was performed with the original spectra, if necessary after the subtraction of strong water bands. This allowed the position of peak maxima to be determined with a precision of better than 0.1 cm^{-1}. In the case of spectra with weak absorption bands, resolution enhancement techniques such as Fourier self-deconvolution (17) were applied after baseline subtraction with the parameters bandwidth, 22–28 cm^{-1}; resolution enhancement factor, 1.2–1.4; and Gauss/Lorentz ratio, 0.55. In the case of overlapping bands, curve fitting was applied using a modified version of the CURFIT procedure written by D. Moffat (National Research Council, Ottawa, Canada). An estimation of the number of band components was obtained from deconvolution of the spectra, curve fitting was then applied within the original spectra after the subtraction of baselines resulting from neighboring bands. Similar to the deconvolution technique, the band shapes of the single components are superpositions of Gaussian and Lorentzian band shapes. Best fits were obtained by assuming a Gauss fraction of 0.55–0.6. The CURFIT procedure measures the peak areas of single-band components and after statistical evaluation determines the relative percentages of primary structure involved in secondary structure formation. For P5, relative levels of α-helical structure (1650–1655 cm^{-1}) and β-sheet structures (1625–1640 cm^{-1}) were computed and are shown in Table 2.

FTIR analysis of phospholipid phase transition properties. Using FTIR spectroscopy, the effect of P5 on the phase transition properties of phospholipid was investigated. To give a final peptide concentration of 1 mM, P5 was solubilized in suspensions of SUVs formed from DMPG, DMPC, or DMPE, which were prepared as described above. As controls, SUVs formed from DMPG, DMPC, or DMPE alone were prepared as described above. All samples were then placed in a calcium fluoride cuvette, separated by a 12.5-μm-thick Teflon spacer and subjected to automatic temperature scans with a heating rate of 3°C 5 min^{-1} within the temperature range 0 to 60°C. For every 3°C interval, 50 interferograms were accumulated, apodized, Fourier transformed, and converted to absorbance spectra (18) (Figs. 1 and 2). These spectra monitored changes in the $\beta \leftrightarrow \alpha$ acyl chain melting behavior of phospholipids with these changes determined as shifts in the peak position of the symmetric stretching vibration of the methylene groups, $\nu_s(CH_2)$, which is known to be a sensitive marker of lipid order. The peak position of $\nu_s(CH_2)$ lies at 2850 cm^{-1} in the gel phase and shifts at a lipid-specific temperature T_c to 2852.0–2852.5 cm^{-1} in the liquid crystalline state.

TABLE 2

Relative Levels of P5 Secondary Structure in the Presence of Lipid

Secondary structural type	DMPC		DMPE		DMPG	
	pH		pH		pH	
	5	9	5	9	5	9
α-Helix (%)	50	58	58	46	44	48
β-Sheet (%)	50	43	42	54	55	51

RESULTS AND DISCUSSION

FTIR conformational analyses of P5, in the presence of membranes formed from DMPC, DMPE, or DMPG, at either pH 5 or pH 9 showed that the peptide adopted high levels of α-helical structure, which ranged between 44 and 58% (Table 2).

In contrast to aqueous P5 (12), these levels of α-helicity showed only minor changes (circa 10%) when pH conditions were moved from strongly acidic to strongly alkaline. These results clearly show that the PBP5 C-terminal sequence has a high propensity to adopt amphiphilic α-helical structure in the presence of lipid but it would appear that these levels show no significant response to major changes in pH.

FTIR lipid-phase transition analysis showed that the presence of P5 led to an increase in the fluidity of DMPC membranes, which was accompanied by a strong decrease in the T_c of the lipid, of the order of

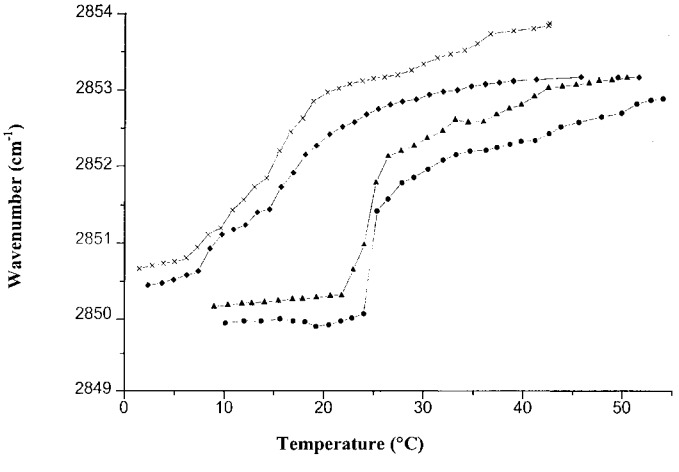

FIG. 1. The effect of P5 on the phase transition properties of DMPC. At both pH 5 (●) and pH 9 (▲), the T_c of DMPC was recorded as 25°C. However, in the presence of P5, the T_c of the lipid had decreased by approximately 15°C at both pH 5 (×) and pH 9 (◆).

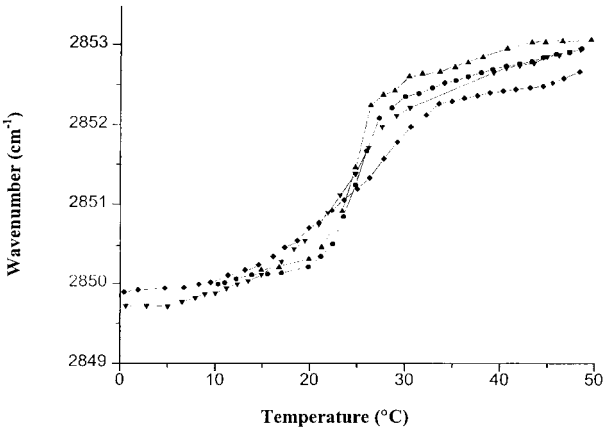

FIG. 2. The effect of P5 on the lipid-phase transition properties of DMPG. At both pH 5 (●) and pH 9 (▲), the T_c of DMPG was recorded as 25°C. However, in the presence of P5, the DMPG gel to liquid crystalline transition occurred over the temperature range 20 to 30°C at both pH 5 (◆) and pH 9 (▼).

15°C at each pH examined (Fig. 1). Similar results were recorded for the effect of P5 on DMPE membranes with the presence of the peptide leading to a decrease in the T_c of the lipid (45°C) of the order of 5°C at both pH 5 and pH 9 (data not shown).

In contrast to DMPC and DMPE, the presence of P5 led to a broadening of the T_c of DMPG membranes (25°C) with phase transition occurring over a temperature range (20 to 30°C) accompanied by an increases in gel-phase fluidity and decreases in liquid crystalline phase fluidity at each pH examined (Fig. 2). This form of phase transition shows similarities to that of some cholesterol–lipid systems (19) and implies that the presence of P5 leads to changes in the hydrocarbon chain packing of DMPG membranes, which results in fluidization of the gel phase and rigidification of the liquid crystalline phase.

The ability of P5 to induce these changes in lipid-phase transition properties suggest that the peptide has the capacity to penetrate both anionic and zwitterionic membranes and to interact with their acyl chain region. Furthermore, the generally high levels of these changes suggest deep levels of membrane penetration by P5 and a major role for hydrophobic forces. Taken with P5's high propensity to adopt amphiphilic α-helical structure, our results strongly support the view that the ability of the PBP5 C-terminal sequence to interact with membranes is related to its ability to adopt such structure. Nonetheless, our results also indicate that the ability of P5 to induce changes in the T_c and fluidity of membranes shows no significant response when pH conditions are moved from strongly acidic to strongly alkaline (Figs. 1 and 2). When these results are taken with the results of our FTIR conformational analyses of P5 (Table 2), they suggest that neither the levels of α-helicity adopted by the PBP5 C-terminal region in the presence of membranes nor the levels of interaction shown by the region with membranes is significantly affected by changes in pH. Based on these results, it would appear that C-terminal conformational change is unlikely to play a significant role in the pH dependency of the membrane anchoring mechanism of PBP5. This would support the suggestion that changes in the levels of membrane anchoring shown by PBP5 and that the interconversion of the urea-resistant and -susceptible forms of the protein may be related to conformational changes in the protein's ectomembranous domain (13, 14).

REFERENCES

1. Phoenix, D. A., and Harris, F. (1998) Amphiphilic α-helices and lipid interactions. *In* Protein Targeting and Translocation (Phoenix, D. A., Ed.), pp. 19–36, Portland Press, London, UK.
2. Phoenix, D. A., and Harris, F. (1995) The membrane interactive properties of the low molecular weight penicillin-binding proteins. *Biochem. Soc. Trans.* **2,** 976–980.
3. Phoenix, D. A., and Pratt, J. M. (1990) pH-induced insertion of the amphiphilic α-helical anchor of *Escherichia coli* penicillin binding protein 5. *Eur. J. Biochem.* **190,** 365–369.
4. Phoenix, D. A., and Harris, F. (2001) A study on the membrane binding of *Escherichia coli* penicillin-binding protein 5. *Biol. Membr.* **18,** 38–41.
5. Roberts, M. G., Phoenix, D. A., and Pewsey, A. R. (1997) An algorithm for the detection of surface-active α-helices with the potential to anchor proteins at the membrane interface. *CABIOS* **13,** 99–106.
6. Pewsey, A. R., Phoenix, D. A., and Roberts, M. G. (1996) Monte Carlo analysis of potential C-terminal membrane interactive α-helices. *Protein Pept. Lett.* **3,** 185–192.
7. Phoenix, D. A. (1990) Investigation into structural features of the *Escherichia coli* penicillin-binding protein 5 C-terminal anchor. *Biochem. Soc. Trans.* **18,** 948–949.
8. Jackson, M. E., and Pratt, J. M. (1988) Analysis of the membrane-binding domain of penicillin-binding protein 5 of *Escherichia coli. Mol. Microbiol.* **2,** 563–568.
9. Jackson, M. E., and Pratt, J. M. (1987) An 18 amino acid amphiphilic helix forms the membrane anchoring domain of the *Escherichia coli* penicillin binding protein 5. *Mol. Microbiol.* **1,** 23–28.
10. Pratt, J. M., Jackson, M. E., and Holland, I. B. (1986) The C-terminus of penicillin-binding protein 5 is essential for localisation to the *Escherichia coli* inner membrane. *EMBO J.* **5,** 2399–2405.
11. Harris, F., Demel, R. A., Phoenix, D. A., and De Kruijff, B. (1998) An investigation into the lipid interactions of peptides corresponding to the C-terminal anchoring domains of *Escherichia coli* penicillin-binding proteins 4, 5 and 6. *Biochim. Biophys. Acta* **1415,** 10–22.
12. Siligardi, G., Harris, F., and Phoenix, D. A. (1997) α-Helical conformation in the C-terminal anchoring domains of *Escherichia coli* penicillin-binding proteins 4, 5 and 6. *Biochim. Biophys. Acta* **1329,** 278–284.
13. Harris, F. (1998) An investigation into the membrane interactive properties of *Escherichia coli* low molecular mass penicillin-binding proteins, Thesis, Univ. Central Lancashire, Preston, UK.

14. Phoenix, D. A., and Pratt, J. M. (1993) Membrane interaction of *Escherichia coli* penicillin-binding protein 5 is modulated by the ectomembranous domain. *FEBS Lett.* **322,** 215–218.

15. Broome-Smith, J. K., Ionnidis, I., Edelman, A., and Spratt, B. G. (1988) Nucleotide sequences of the penicillin-binding protein 5 and 6 genes of *Escherichia coli*. *Nucleic Acids Res.* **16,** 1617.

16. Keller, R. C., Killian, J. A., and De Kruijff, B. (1992) Anionic phospholipids are essential for α-helix formation of the signal peptide of PhoE upon interaction with phospholipid vesicles. *Biochemistry* **31,** 1672–1677.

17. Kauppinen, J. K., Moffat, D. J., Mantsch, H. H., and Cameron, D. G. (1981) Fourier self-deconvolution—A method for resolving intrinsically overlapped bands. *Appl. Spectrosc.* **35,** 271–276.

18. Brandenburg, K., Kusomoto, S., and Seydel, U. (1997) Conformational studies of synthetic lipid A analogues and partial structures by infrared spectroscopy. *Biochim. Biophys. Acta* **1329,** 183–201.

19. Ohvo-Rekila, H., Ramstedt, B., Leppimaki, P., and Slotte, J. P. (2001) Cholesterol interactions with phospholipids in membranes. *Prog. Lipid Res.* **41,** 66–97.