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# Equilibrium and non-equilibrium conformations of peptides in lipid bilayers

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**Abstract**

A synthetic, hydrophobic, 27-amino-acid-residue peptide 'K27', modelled on the trans-membrane domain of the slow voltage-gated potassium channel, IsK, has been incorporated into a lipid bilayer and its conformational properties studied using FT–IR spectroscopy. The conformation following reconstitution is found to be dependent on the nature of the solvent employed. When the reconstitution is conducted by solvent evaporation from a methanol solution, aggregates comprised of β-strands are stabilised and their concentration is essentially invariant with time. By contrast, when trifluoroethanol is used, the initial conformation of the peptide is α-helical. This then relaxes to an *equilibrium state* between α-helices and β-strands. The α-helix-to β-strand conversion rate is relatively slow, and this allows the kinetics to be studied by FT–IR spectroscopy. The reverse process is much slower but again can be demonstrated by FT–IR. Thus, it appears that a true equilibrium structure can only be achieved by starting with peptide in the α-helical conformation. We believe this result should be of general validity for hydrophobic peptide reconstitution. The implications for conformational changes in membrane proteins are discussed. © 1997 Elsevier Science B.V.

*Keywords:* FT–IR spectroscopy; Secondary structure; Conformational transition; Bilayers; Peptides

## 1. Introduction

The reconstitution of peptides into lipid bilayer membranes by detergent dialysis or solvent removal from solutions of lipid and peptide is commonly used to obtain model membrane systems for biochemical or biophysical study [1–3]. Whether different solvents and reconstitution methods affect the conformation of peptides in the membrane has rarely been questioned. However, we have found that a synthetic, 27-mer peptide, 'K27' with primary structure **KLEALYILMVLGFFGFFTLGIMLSYIR** modelled on the transmembrane domain of the slow voltage gated potassium channel protein IsK [4], adopts conformations in a lipid bilayer which are sensitive to the solvent employed in reconstitution.

In this paper, we investigate the possible reasons for the different conformations adopted by the peptide in the same lipid environment when different organic solvents were used in the peptide incorporation. This leads to the development of methodology for incorporating hydrophobic peptide in lipid bilayer in its equilibrium structure, which has practical significance for biophysical studies of peptides.

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## 2. Materials and methods

### 2.1. Peptide synthesis

K27 is modelled on the transmembrane sequence (residues 45–67) of the IsK potassium ion channel protein [4]. A few polar residues (K, L, E and R) at the N and C termini are retained so as to anchor the peptide to the bilayer surfaces. It was synthesised on a 0.1 g scale using an automated MilliGen/Biosearch 9050 solid-phase synthesiser. Fmoc chemistry was used for amino acid coupling. Couplings were controlled by counterion distribution monitoring to achieve not less than 99% coupling efficiency. After completion of the synthesis, the resin was washed extensively with 150 ml portions of *t*-amyl alcohol, glacial acetic acid and *t*-amyl alcohol, followed by 300 ml of diethyl ether. The peptide was cleaved from the resin using a cleavage mixture of 95% trifluoroacetic acid, 2.5% phenol and 2.5% ethanedithiol. After rotary evaporation to remove the trifluoroacetic acid, the residue was dissolved in hexafluoroisopropanol, precipitated with diethyl ether and washed six times by re-suspension in diethyl ether. The vacuum-dried precipitate was dissolved in hexafluoroisopropanol (1 ml), diluted to 100 ml with deionised water and freeze dried. Further purification of the peptide by reverse phase HPLC was attempted but found to be impracticable due to the extreme hydrophobicity of the peptide. Amino acid sequence analysis using both solid and liquid phase strategies indicated $> 80\%$ purity in relation to deletion peptides. Further information on the synthesis and characterisation of K27 is published elsewhere [5].

## 3. Sample preparation

To prepare samples, an appropriate volume of a dimyristoylphosphatidylcholine DMPC (purchased from Lipid Products, Surrey, England) stock solution was evaporated under a steam of nitrogen gas, then dried under vacuum for several hours to remove any traces of the solvent. A solution of K27 (1mg/ml) in either trifluoroethanol or methanol was added to the dry DMPC to produce peptide/lipid solutions at a specific peptide/lipid ratio $R_P$. The solvent was then evaporated under a stream of nitrogen gas and the sample further dried under vacuum for at least 10 h. Finally, the peptide/lipid mixtures were hydrated with $D_2O$ at a $D_2O$/lipid ratio, $R_W$, of 25 and incubated at 40°C for 10 h. During incubation, the samples were mixed occasionally by centrifugation. It was found that the mixing process decreases the time necessary for the peptide to reach its structural equilibrium in the bilayers.

## 4. FT–IR spectroscopy

FT–IR spectroscopy was used to determine the secondary structure of the peptide. All the spectra were recorded at 40°C on a Perkin-Elmer 1760X FT–IR Spectrometer controlled by a PC with data processing software. A home-made thermostatted liquid cell with $CaF_2$ windows and pathlength about 50 μm was used. Temperature was controlled to within $\pm 0.1$°C by circulating water from a cryostat (Colora WK 3, West Germany). Each spectrum is an accumulation of 8 scans with a resolution of 4 $cm^{-1}$. After Fourier transformation, the spectra were analysed using a band-fitting program and the proportions of α-helix and β-strand were estimated using the relative integrated intensities of the corresponding amide I bands.

## 5. Results and discussion

### 5.1. Conformation of the peptide in the lipid bilayers

The IR spectra of K27, reconstituted into DMPC bilayer after 10 h incubation using either trifluoroethanol or methanol, are shown in Fig. 1. Spectrum (a), trifluoroethanol as solvent, shows two major IR bands in the amide I region (1700–1600 $cm^{-1}$). The bands at 1655 $cm^{-1}$ and 1626.5 $cm^{-1}$ are assigned, respectively, to α-helical and β-strand conformation [6–8]. The proportions of the α-helical and β-strand structures is 20 and 80 per cent, estimated from band-fitting analysis. Spectrum (b), methanol as solvent, shows a predominant amide I band at frequency 1625.5 $cm^{-1}$, corresponding to β-strand conformation. On both spectra a and b, there is a minor amide I band at 1648 $cm^{-1}$, which is assigned to random structure [9]. The broad band

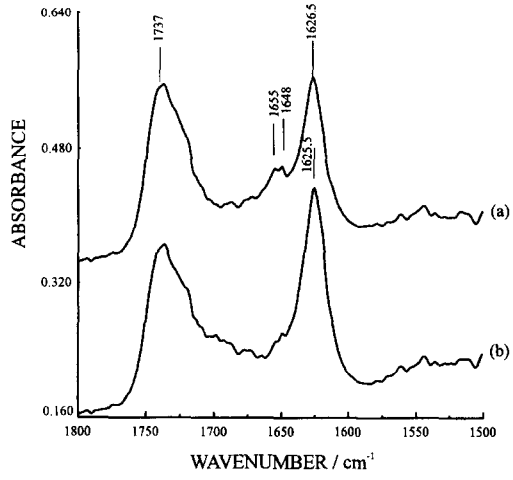


Fig. 1. IR spectra of K27/DMPC bilayers prepared using trifluoroethanol (a) and methanol (b) at $R_P = 0.05$, $R_W = 25$ and $T = 40°C$. The bands at 1655 $cm^{-1}$, 1626.5 $cm^{-1}$ and 1648 $cm^{-1}$ are assigned to α-helical, β-strand and random coil conformations respectively.

at 1737 $cm^{-1}$ is associated with the ester carbonyl stretch in the lipid chains [10].

The conformation of K27 in trifluoroethanol and methanol solutions is found to be mainly α-helical and β-strand, respectively (Fig. 2a, b). The fact that 80 per cent of the peptide incorporated into lipid bilayers using trifluoroethanol as solvent (Fig. 1a) is present in the β-strand conformation demonstrates a significant conversion from α-helical to β-strand conformation. This raises the interesting question as to whether 80% β-strand corresponds to the equilibrium state of the peptide under these conditions. The fact that the conformation of K27, starting with methanol as the solvent (Fig. 1b), is essentially 100 per cent β-strand suggests that this may not necessarily be so. It could, however, be that conversion from the β-strand to α-helix within the lipid bilayer is a very slow process so that the equilibrium is not attainable. To distinguish between the two possibilities, we have investigated the rates of the conversion processes.

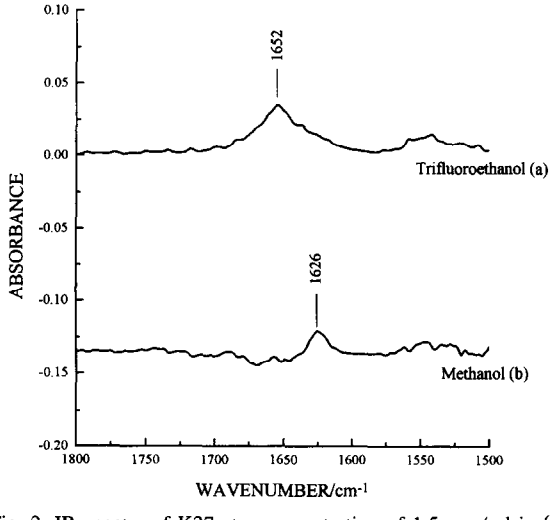


Fig. 2. IR spectra of K27 at a concentration of 1.5 mg/ml in (a) trifluoroethanol and (b) methanol solutions.

## 6. Kinetics of α-to-β conformational transitions

### *6.1. The α-to-β transition*

The kinetics of the α-helical to β-strand conformational transition was studied using FT–IR spectroscopy to monitor the changes in the relative proportion of the two conformations with respect to time immediately following reconstitution. A K27/DMPC mixture at $R_P = 0.07$, prepared from trifluoroethanol, was hydrated with $D_2O$ at $R_W = 25$ (time $t = 0$). The bilayers were then mixed extensively by centrifugation, sealed between $CaF_2$ IR windows with vacuum gel, and maintained at 40°C throughout the whole period of the measurements (103 h). IR spectra measured at various times are shown in Fig. 3. The peptide was initially in the α-helical conformation, judged from a major amide I band at 1656 $cm^{-1}$ in the spectrum taken after 30 min. The band at 1673.5 $cm^{-1}$ arises from residual trifluoroacetic acid (TFA), which is often found in synthetic peptides [11]. The intensity of the α-helical band is seen to decreases with time. Simultaneously, a band at 1626.5 $cm^{-1}$, corresponding to the β-strand conformation, is seen to increase gradually with time. The changes of α-helix concentration as a function of time has been further analysed by plotting the reciprocal concentration of the α-helix vs time (Fig. 4). The linear nature of the plot suggests that the conformational transition is probably a second order process with a rate constant of 0.067 $M^{-1}s^{-1}$.

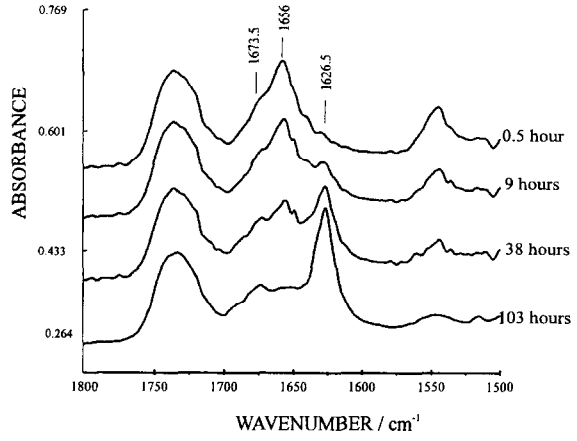


Fig. 3. IR spectra of K27/DMPC, prepared using trifluoroethanol ($R_P = 0.07$, $R_W = 25$ and $T = 40°C$), measured as a function of time following reconstitution.

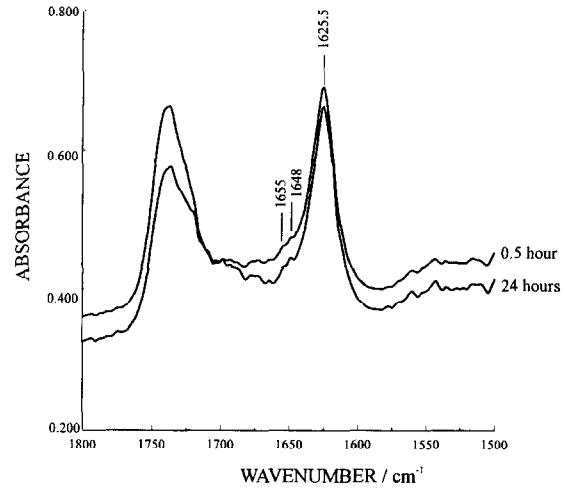


Fig. 5. IR spectra of K27/DMPC bilayer, prepared using methanol as solvent ($R_P = 0.05$, $R_W = 25$ and $T = 40°C$), measured at different times following reconstitution.

### 6.2. β-to-α transition

The kinetics of the β-strand to α-helical conformational transition were also investigated. A sample of *K27*/DMPC bilayers at $R_P = 0.05$, $R_W = 25$ was prepared using methanol as the solvent. Fig. 5 shows the spectra measured after 30 min and 24 h following sample hydration. The closely similar spectral features indicates there is no significant conformational change over 24 h. Compared to the rate of the α-helical to β-strand conformational transition, the β-strand to α-helix transition appears to be a much slower.

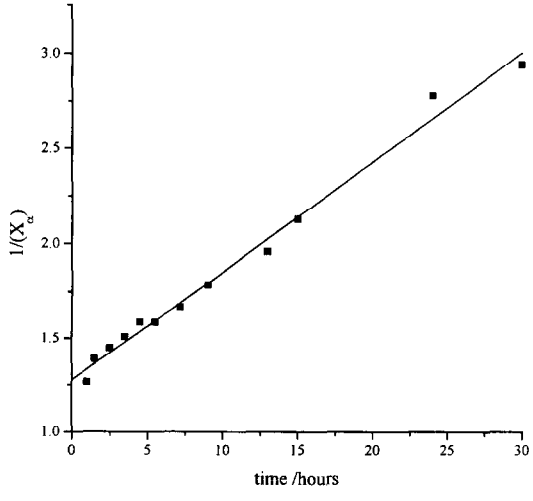


Fig. 4. Plot showing reciprocal concentrations of the α-helical conformer of K27 in DMPC bilayers as a function of time.

The reason for the different kinetics is believed to be due to the nature of the two structures. α-Helices exist in the lipid bilayer either as monomer or as small, weakly-associated aggregates since K27 is a hydrophobic peptide. The interactions between the hydrophobic helices are mainly due to weak van der Waal's forces. In contrast, β-strand conformers are present in aggregates involving relatively strong intermolecular hydrogen bonds. In speculating about the mechanism of α-helix to β-strand conformational conversion, it is likely that two helices come together as established by Engelman and coworkers with a number of membrane proteins [12,13]. With K27, the helices then unfold to form a β-strand dimer in the rate determining step since the kinetic behaviour can be analysed as a second order process (Fig. 4). Because there are free hydrogen bonding groups in the ends of β-strand dimers, more α-helices can then join the β-strand dimers to form bigger β-aggregates (β-sheets). Regarding the mechanism of the β-strand to α-helix conversion, it is likely that a single β-strand needs to dissociate from an aggregate before it can undergo a conformational transition. The probability of such event is very small as the dissociations occurring in any positions other

than at the ends of a β-sheet would just produce two small β-sheets.

## 7. Demonstration of the achievement of a true equilibrium

The conformation of K27 reconstituted into lipid bilayers is found to be a complex function of the concentration of the peptide. Studies of K27 in egg phosphatidylcholine bilayers at peptide/lipid molar ratios ($R_P$) in the range 0.01–0.10 and hydration (moles of water/lipid, $R_W$) of 25 have shown that at $R_P$ 0.01, only 5% of the peptide is in the β-strand conformation whilst at $R_P = 0.10$, 23% is in this conformation. This concentration dependence can be analysed to provide an insight into the mechanism of the aggregation process. Full details will be given in a forthcoming publication (A. Aggeli, N. Boden, P.F. Knowles and Y. Cheng in preparation). Here the objective is to demonstrate that a true equilibrium between the α-helical and β-strand conformers is established. If this is the case, then decreasing the peptide concentration should result in an increase in the α-helical conformation. This has been demonstrated by mixing equal amounts of two samples, one having $R_P = 0.00$ and the other $R_P = 0.03$. After 4 days incubation at 40°C, a slight but reproducible decrease in the intensity of the β-strand band (1625.5 $cm^{-1}$) and increase in the intensity of the α-helical band (1654 $cm^{-1}$) was observed (Fig. 6). These observations demonstrate that the aggregation process is reversible and that a true equilibrium state is realisable in samples prepared by solvent evaporation. By contrast, similar mixing experiments using β-strand aggregates incorporated by dialysis show that they are invariant with time. In this case, the β-strand aggregates are pre-formed in the solvent before being incorporated into the bilayer and equilibrium cannot be established.

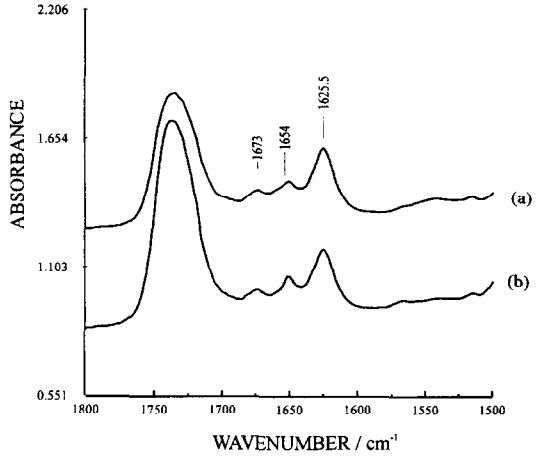


Fig. 6. IR spectra of K27/DMPC bilayers ($R_P = 0.03$, $R_W = 25$ and $T = 40°C$) (spectrum a), and four days after dilution with an equal volume of DMPC bilayers ($R_W = 25$) (spectrum b).

## 8. Conclusions

The kinetics of the α-helix to β-strand and β-strand to α-helix conversions have been studied. The process of β-strand to α-helix conversion is a much slower process, which suggests that once a peptide has been incorporated into a β-strand conformation, it is essentially 'permanently' locked into that state. On the other hand, the process of α-helix to β-strand conversion is faster, which is of practical significance as it can be used as a basis for hydrophobic peptide reconstitution into bilayers to achieve an equilibrium state. We have demonstrated in the present paper that the hydrophobic peptide K27 can be incorporated into the membrane in an equilibrium manner by using trifluoroethanol as a solvent. Our results have practical significance for the preparation of peptide/lipid samples for biochemical and biophysical studies. For instance, only an equilibrium situation permits the study of factors modulating a peptide conformation in lipid bilayers.

Our results suggest that care should be taken in accepting the functional relevance to the protein of studies on the conformation of peptides modelled on the transmembrane domains of membrane proteins even when these are made in membrane-like environments. For example current opinion suggests that the core region of voltage-gated ion channels involves β-barrel structures [14] though spectroscopic studies of model peptides for these channels in lipid vesicles suggest a predominantly α-helical conformation [15]. Other examples are given in a recent review of peptide models for membrane channels [16] and include further discussion of peptides modelled on the IsK potassium channel and the IsK protein itself.

Our findings also have medical implications with respect to amyloid diseases (Alzheimer's disease and prion-related diseases such as scrapie). A conformational transition from α-helix to β-strand in a membrane precursor protein has been implicated in Alzheimer's disease [17,18] and scrapie [19] leading to the deposition of β-amyloid peptide plaques which are highly insoluble. Shen and Murphy [18] have studied the mechanism of self-assembly of β amyloid peptide in different solvents to give fibrillar structures and suggest from their results that formation of a β-sheet oligomer is a controlling step in plaque formation and that assembly by dilution from solvent into aqueous medium depends on the solvent used. These conclusions are strikingly similar to the conclusions drawn from our studies with K27 in membranes. Although secondary sequence predictions of β-amyloid peptide in membranes have been made, and theoretical models proposed involving different assemblies of β-hairpin and α-helix conformational states which might inter-convert [20], direct studies of the mechanism of self-assembly of β-amyloid peptides in membranes have not yet been made. The results we have presented indicate that once a conversion of α-helix to β-sheet has occurred in the β-amyloid precursor protein, there would be a kinetic barrier which would limit the reverse process and thus lead to insoluble plaques.

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## References

[1] O.G. Mouritsen and M. Bloom, Ann. Rev. Biophys. Biomol. Struct., 22(1993) 145.
[2] K.S. Åkerfeldt, J.D. Lear, Z.R. Wasserman, L.A. Chung and W.F. DeGrado, Acc. Chem. Res., 26 (1993) 191.
[3] Y-P. Zhang, R.N.A.H. Lewis, G.D. Henry, B.D. Sykes, R.S. Hodges, and R.N. McElhaney, Biochemistry, 34 (1995) 2348.
[4] T. Takumi, H. Ohkubo, and S. Nakanishi, Science, 242 (1988) 1042.
[5] A. Aggeli, N. Boden, Y.L. Yang, J.B.C. Findlay, P.F. Knowles, P. Kovatchev, P.J.H. Turnbull, L. Horvath and D. Marsh Biochemistry, 35 (1996) 16213.
[6] T. Miyazawa and E.R. Blout, J. Am. Chem. Soc., 83 (1961) 712.
[7] W.K. Surewicz, H.H. Mantsch and D. Chapman, Biochemistry, 32(1993), 389.
[8] S. Krimm and J. Bandekar, Adv. Protein Chem., 38 (1986) 181.
[9] W.K. Surewicz and H.H. Mantsch, Biochim. Biophys. Acta, 952 (1988) 115.
[10] S.F. Bush, H. Levin, and I.W. Levin, Chem. Phys. Lipids, 27 (1980) 101.
[11] B. Kleffel, R.M. Garavito, W. Baumeister and J.P. Rosenbusch, EMBO J., 4 (1985) 1589.
[12] J-L. Popot and D.M. Engelman, Biochemistry, 29 (1990) 4031
[13] B.D. Adair and D.M. Engelman, Biochemistry, 33 (1994) 5539
[14] S.R. Durrell and H.R. Guy, Biophys. J., 62 (1992) 238.
[15] P.I. Haris, B. Ramesh, M.S.P. Sansom, I.D. Kerr, K.S. Srai and D. Chapman, Protein Eng., 7 (1994) 255.
[16] D. Marsh, Biochem. J., 315 (1996) 345.
[17] M.G. Zagorski and C.J. Barrow, Biochemistry, 31 (1992) 5621.
[18] C-L. Shen and R.M. Murphy, Biophys. J., 69 (1995) 640.
[19] K-M. Pan, M. Baldwin, J. Nguyen, M. Gasset, A. Serban, D. Groth, I. Mehlhorn, Z-W. Huang, R.J. Fletterick, F.E. Cohen and S.B. Prusiner, Proc. Natl. Acad. Sci. USA., 90 (1993) 10962.
[20] S.R. Durell, H.R. Guy, N. Arispe, E. Rojas and H.B. Pollard, Biophys. J., 67 (1994) 2137.