FEBS Letters 349 (1994) 371–374

FEBS 14318

The conformational analysis of a synthetic S4 peptide corresponding to a voltage-gated potassium ion channel protein

Parvez I. Haris*, Bala Ramesh, Stephen Brazier, Dennis Chapman

Department of Protein and Molecular Biology, Royal Free Hospital School of Medicine, University of London, Rowland Hill Street, London, NW3 2PF, UK

Received 17 June 1994

Abstract

The S4 region of the *Drosophila Shaker* voltage-gated K^+ channel has been proposed to function as a voltage-sensor. We have synthesised a peptide corresponding to this S4 region. Structural studies on the S4 peptide were conducted using Fourier transform infrared (FTIR) spectroscopy. Spectra were obtained for the peptide dissolved in aqueous solution, in trifluoroethanol solvent and also after reconstitution into lipid bilayers and micelles. The peptide in trifluoroethanol adopts an α-helical conformation which is in good agreement with the results of a recent 2D NMR study on the structure of a S4 peptide corresponding to the rat brain sodium channel [(1989) FEBS Lett. 257, 113–117]. A predominantly α-helical structure is also observed when the S4 peptide is present in aqueous lysophosphatidylcholine micelles, in dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol lipid bilayers. In contrast to this, the S4 peptide in aqueous solution is in a random coil conformation. The coil-to-helix transition observed for the S4 peptide upon its transfer from aqueous solution to lipid membrane indicates that it has a high degree of conformational flexibility and can undergo large changes in its structure in response to its environment. This may have important implications for its role in the voltage activation process during which the S4 peptide has been postulated to, at least partially, move from a lipid bilayer to an aqueous extracellular media [(1992) Biophys J. 62, 238–250]. The results of our study lend support to such a model.

Key words: Protein conformation; FTIR spectroscopy; S4 domain; Voltage sensor; Potassium channel

1. Introduction

Voltage-gated ion channels are integral membrane proteins which conduct ions across biomembranes and are responsible for electrical excitability. Voltage-gated ion channels are thought to be constructed from four similar domains with each domain consisting of six membrane spanning segments, S1 to S6 (for reviews, see [1–3]). The relatively small size of voltage-gated K^+-channels makes them particularly attractive for structure–function analysis. Recent studies employing site-directed mutagenesis in conjunction with electrophysiological measurements have identified the functional role of several regions of this protein [2,3]. The most recent advance has been the identification of the sequence which constitutes the ion-selective pore [4–6]. Prior to this, a highly conserved S4 transmembrane domain has been suggested to have a role in sensing the transmembrane electric potential [7,8]. Mutations in the S4 segment of voltage-dependent ion channels have been shown to affect both voltage-sensing and gating currents [7–12]. The S4 segment contains a positively charged amino acid residue (lysine or arginine) at every third position. Mutagenesis studies have shown that these basic residues in the S4 region play a central role in the voltage-dependence of activation of K^+ channels. Further support indicating that S4 is involved in voltage sensing comes from experiments in which S4 domains are swapped. In these situations the voltage-dependence is conferred largely by the substituted S4 domain [13,14].

Although mutagenesis studies have provided valuable information on the possible functional role of the S4 sequence, little is known about its structure. Recently, some studies have been directed towards obtaining a structural understanding of the S4 sequence. For example, the solution structure of a synthetic peptide corresponding to the S4 sequence of the rat brain Na^+ channel has been determined using 2D NMR spectroscopy [15]. The ion conductance property of a synthetic Na^+ channel S4 peptide in planar lipid bilayers has also been investigated [16]. While, some structural data are available for the S4 sequence of the Na^+ channel, little or no data is as yet available for the corresponding sequence of the K^+ channel. In the present study we employ FTIR spectroscopic measurements for conformational analysis of a synthetic peptide corresponding to the S4 sequence of the *Shaker Drosophila* K^+ channel. FTIR spectroscopy is a useful technique for the structural analysis of peptides and proteins in an aqueous lipid bilayer environment (for a recent review see [17]).

*Corresponding author. Fax: (44) (71) 431 8107.

Abbreviations: FTIR, Fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; CD, circular dichroism spectroscopy; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; LPC, lyso-phosphatidylcholine; TFE, trifluoroethanol; MBHA, 4-methyl benzhydrylamine.

SSDI 0014-5793(94)00704-7

2. Materials and methods

2.1. Materials

Dimyristoyl L-α-phosphatidyl choline (DMPC), dimyristoyl L-α-phosphatidyl-DL-glycerol (DMPG) and Lyso phosphatidyl choline (LPC), were purchased from Sigma Chemicals Ltd., UK. Protected amino acids were obtained from Novabiochem, UK.

2.2. Peptide synthesis and purification

The S4 peptide was synthesised on an automated peptide synthesiser (Rainin PS3, Protein Technologies, Inc.) by a stepwise solid-phase procedure [18] using Nα-9-fluorenylmethyloxycarbonyl (Fmoc) protecting group strategy [19]. All the arginines had their side chains protected with 2,2,5,7,8-Pentamethylchroman-6-sulphonyl group and were double coupled throughout the synthesis. The Rink amide MBHA resin was used to generate the peptide with C-terminal amides and their N terminal acetylated on completion of synthesis. The peptide was initially cleaved from the resin using 10% trifluroacteic acid (TFA) in dichloromethane and final cleavage achieved with 95% aqueous TFA in the presence of scavengers. The crude peptide was purified on a semi-preparative reverse-phase HPLC column TSK ODS 120T column (300 mm × 7.8 mm ID) using 0.1% trifluoroacetic acid and acetonitrile as eluents. One major peak was observed when the eluate was monitored at 220 nm. The sequence of the 20 amino acid S4 peptide synthesised is as follows:

CH_3-**ILRVIRLVRVFRIFKLSRHS**-NH_2

The peptide was further characterised by amino acid analysis and the correct molecular weight was confirmed by laser desorption mass spectrometry. The peptide was found to be readily soluble in H_2O.

2.3. Spectroscopic measurements

Spectra of the S4 peptide were recorded for samples in aqueous solution, trifluoroethanol (TFE) with 2% ^2H_2O, LPC micelles and in phospholipid bilayers composed of DMPC and DMPG. In order to reconstitute the S4 peptide into phospholipid vesicles, the peptide was dissolved in trifluoroacetic acid and the solvent removed by rotary evaporation to produce a thin film. Phospholipid dissolved in dichloromethane was subsequently added to the peptide film, and once again the solvent removed. Further drying of the thin film of lipid-peptide mixture was carried out under vacuum for 24 h. Finally, ^2H_2O buffered with phosphate (150 mM sodium phosphate, pH 7.4) was added to the lipid-peptide film and mixed thoroughly using a Vortex mixer. The peptide to phospholipid molar ratios used in this study was 1:30.

Infrared spectra were recorded on a 1750 Perkin-Elmer spectrometer equipped with a TGS detector [20]. The spectrometer was continuosly purged with dry air to reduce water vapour absorption in the spectral region of interest. Samples were placed in a Specac cell fitted with a 50 μm Teflon spacer. For each sample 200 scans were signal averaged at a resolution of 4 cm^{-1}. The peptide concentration employed for the FTIR measurements was 10 mg/ml. Absorbance spectra of the peptide were obtained by digital subtraction of the solvent spectrum i.e. either buffered ^2H_2O or 98% TFE. Detailed analysis of the amide I band was carried out using the second-derivative method [20].

3. Results

Analysis of the amide I band in the FTIR spectra of proteins and peptides provide useful information on their secondary structure (for a recent review see [17]). The relationship between the frequency of the amide I band and secondary structure is now well established. The organic solvent TFE is known to enhance intermolecular interactions and therefore has been often used as an α-helical structure-inducing and stabilizing media. Spectra of the S4 peptide were therefore recorded for samples in TFE (Fig. 1). The amide I maximum is centred at 1,654 cm^{-1}. This can be attributed to an α-helical structure. Second-derivative analysis shows the main amide I maximum at 1,655 cm^{-1}. Minor components are revealed at 1,634 cm^{-1} and 1,683 cm^{-1}. These bands are

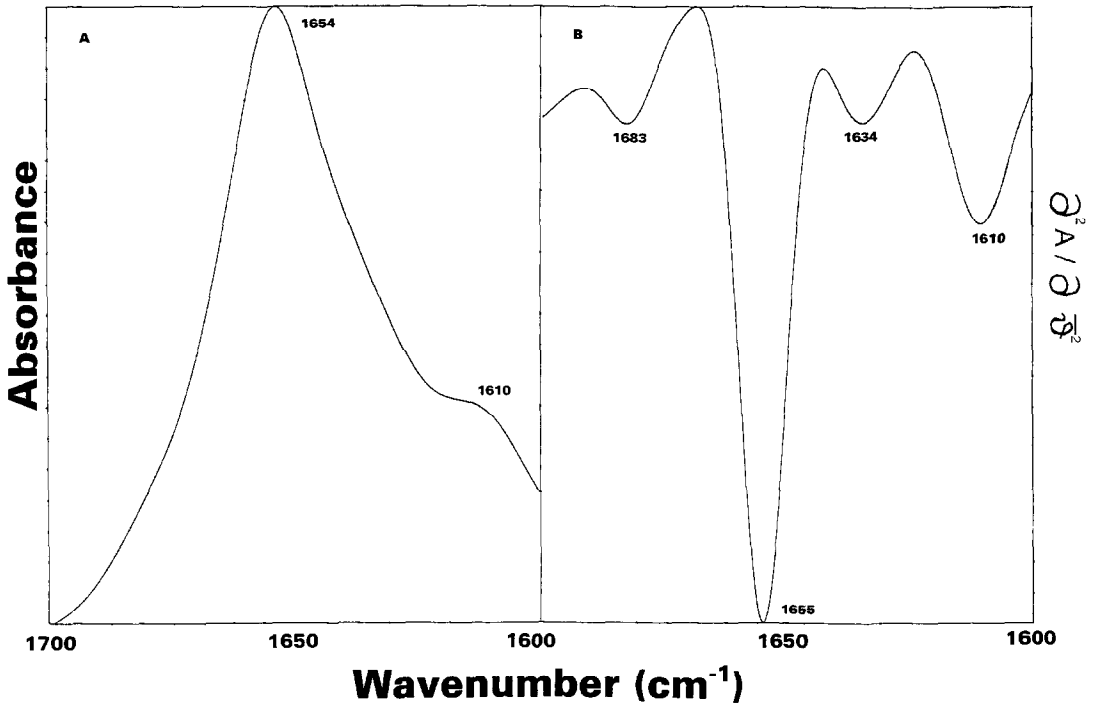

Fig. 1. FTIR absorbance (A) and second-derivative (B) spectra of S4 peptide in trifluroethanol solvent with 2% ^2H_2O. The spectrum was recorded at 30°C.

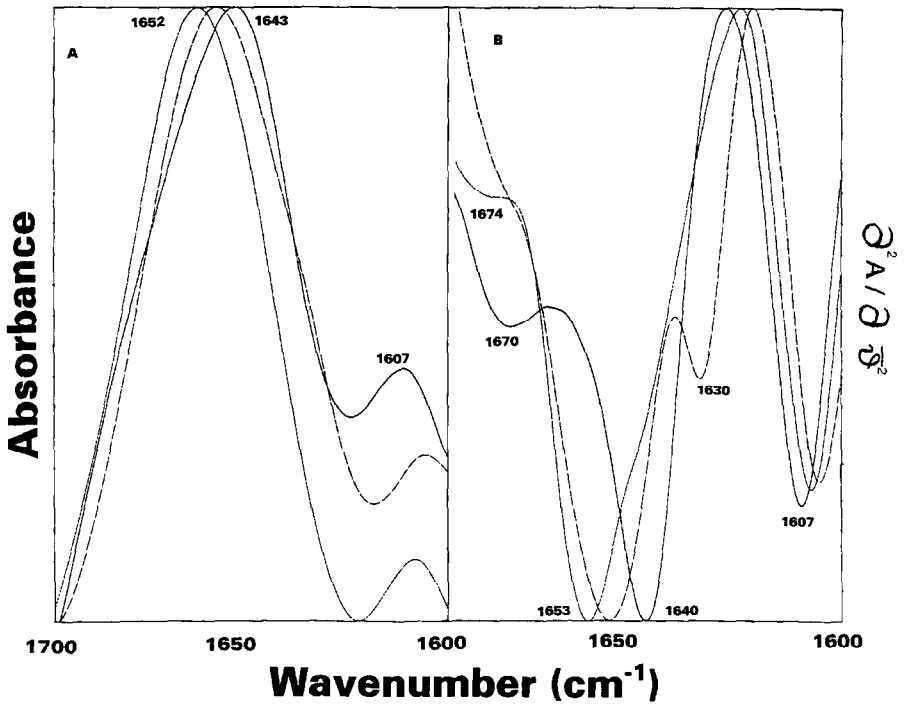

Fig. 2. FTIR absorbance (A) and second-derivative (B) spectra of the S4 peptide in aqueous lysophosphatidyl choline micelles (----); in DMPC vesicles (·····); in aqueous solution (—). All the spectra were recorded for samples in phosphate buffer, (150 mM sodium phosphate, pH 7.4) at 30°C.

weak and may originate from some turn type structure although overlap from β-sheet structure can also occur. The CD spectrum (not shown) displays double minima at 208 nm and 222 nm which is also typical of peptides and proteins in an α-helical conformation. Thus both types of measurement indicate that the S4 peptide has an α-helical structure when the S4 peptide is present in TFE solvent. This result is also in good agreement with the previous 2D NMR study of the S4 peptide corresponding to the rat brain sodium channel [15].

FTIR spectra of the S4 peptide were recorded in aqueous LPC micelles. The FTIR absorbance spectrum (Fig. 2a) shows a maximum at 1649 cm^{-1}. Detailed analysis using the second-derivative method (Fig. 2b) reveals a component of weak intensity at 1,630 cm^{-1} and 1,670 cm^{-1}. These bands may arise from some turn/β-sheet structures. The main amide I band at 1,649 cm^{-1} is consistent with an α-helical structure. Evidence for an α-helical structure for the S4 peptide is also supported by the result of the CD study (not shown).

Fig. 2a also shows the absorbance spectrum of the S4 peptide in aqueous DMPC vesicles. The amide I band is centred at 1,652 cm^{-1}. Detailed analysis using the second-derivative method (Fig. 2b) reveals the main amide I band at 1,653 cm^{-1}. This is consistent with the presence of predominantly α-helical structure for the S4 peptide in DMPC lipid. Similar results were also obtained for the S4 peptide when reconstituted in DMPG phospholipid. Interestingly, raising the temperature above the lipid phase transition temperature of DMPC results in an increase in α-helical structure.

Fig. 2a shows the FTIR absorbance spectrum of the S4 peptide in aqueous solution (pH 7.4). The amide I band is centred at 1,643 cm^{-1}. Detailed analysis of the amide I band using the second-derivative method (Fig. 2b) reveals only one additional component at 1,670 cm^{-1}. The latter band is very weak in intensity, it may originate from turns/bends within the peptide structure. The main amide I maximum of 1,640–1,643 cm^{-1} can be attributed to random coil structure. This assignment is further supported by our CD study which shows a spectrum typical of unordered conformation.

4. Discussion

Mutagenesis results clearly suggest that the S4 sequence play an important role in the conformational change which accompanies gating [3,7–12]. While these studies have provided valuable information about the possible functional role of the S4 sequence, very little hard structural data is available to explain the mechanism by which it achieves this function. Voltage-gated ion channels are large membrane proteins which are as yet not available in sufficient quantities for biophysical studies. This has been a major barrier for carrying out structural studies. One approach towards obtaining some structural information on these proteins is to syn-

thesise peptides corresponding to different domains of its primary sequence which can then be analysed using biophysical techniques. The S4 sequence of the Na^+ channel has been studied in this way using NMR spectroscopy [15]. We have also used this approach to investigate the structure of peptides corresponding to the ion-selective pore of voltage-gated potassium channels [21].

In the absence of structural data various models have been proposed as to how S4 is involved in voltage-sensing [22–25]. In one such model it is postulated that the voltage-dependent conformational change involves a 'helical screw' movement of the charged S4 segment towards the extracellular surface [22]. The S4 is in its 'innermost' position in the protein structure when the channel is in the D_1 deactivated state. Durell and Guy [24] have described how the S4 can move from the D_1 to the open conformation by such an 'helical screw' mechanism. In the open conformation all the positively charged amino acids of S4 are on or near the extracellular surface, forming salt bridges with negatively charged residues in S1, S2, S3 and S5. The model of Durell and Guy [24] indicates that a substantial fraction of the S4 segment moves outside the lipid bilayer in the open state. This requires the peptide to be conformationally flexible, and the transfer from a membrane environment to a hydrophilic aqueous environment is likely to involve some rearrangement in the peptide structure. The results presented here provides information on such conformational changes for the S4 peptide. We have shown that the peptide is predominantly α-helical in TFE solvent, lysophosphatidylcholine micelles and in phospholipid membranes. However, upon transfer to an aqueous solution environment the peptide undergoes a major conformational rearrangement and adopts a random coil structure.

An increase in α-helicity was detected with the peptide in DMPC bilayers at temperatures above the lipid phase transition. We attribute this increase in α-helical structure to greater penetration of the peptide into the lipid bilayer due to the increase in lipid fluidity. We envisage that as the lipid chains crystallize the peptide may be extruded from the lipid bilayer. The results of our FTIR spectroscopic study indicate that the S4 peptide has the conformational flexibility necessary for it to move from the lipid bilayer to the extracellular aqueous media. There is in fact some evidence to indicate that S4 does get exposed to the extracellular medium. Mutating one positive charge (lysine) near the NH_2-terminal of S4 alters the influence of extracellular Ca^{2+} ions but not that of intracellular Mg^{2+} [26]. This is interpreted to signify that this charge is exposed to the extracellular medium. Furthermore, antibodies to the S4 sequence of Repeat I in Na channels appear to bind on the extracellular surface when the membrane is depolarized [27]. Our observation of a helix-coil transition for the S4 sequence is also consistent with a recent theoretical study which suggests that such a major conformational change occurs during the closing and opening of the Na^+ channel [25].

Acknowledgements: We would like to thank the IRC in Biomedical Materials (P.I.H. and D.C) and the SERC (S.B.) for financial support.

References

[1] Catterall, W.A. (1992) Physiol. Rev. 72, S15-S48.
[2] MacKinnon, R. (1991) Curr. Opin. Neurobiol. 1, 14–19.
[3] Pongs, O. (1992) Physiol. Rev. 72, S69–S88.
[4] Yellen, G., Jurman, M.E., Abramson, T. and MacKinnon, R. (1991) Science 251, 939–942.
[5] Hartman, H.A., Kirsch, G.E., Drewe, J.A., Taglialatela, M., Joho, R.H. and Brown, A.M. (1991) Science 251, 942–944.
[6] Yool, A.J. and Schwarz, T.L. (1991) Nature 349, 700–704.
[7] Stuhmer, W., Conti, F., Suzuki, H., Wang, X., Noda, M., Yahagi, N., Kubo, H. and Numa, S. (1989) Nature 339, 597–603.
[8] Papazian, D.M., Timpe, L.C., Jan, Y.N. and Jan, L.Y. (1991) Nature 349, 305–310.
[9] Bezanilla, F., Perozo, E., Papazian, D.M. and Stefani, E. (1991) Science 254, 679–683.
[10] Liman, E.R., Hess, P., Weaver, F. and Koren, G. (1991) Nature 353, 752–756.
[11] Logothetis, D.E., Movahedi, S., Satler, C., Lindpaintner, K. and Nadal-Ginard, B. (1992) Neuron 8, 531–540.
[12] Perzo, E., Santacruz Toloza, L., Stafani, E., Bezanilla, F. and Papazian, D.M. (1994) Biophys. J. 66, 345–354.
[13] Logothetis, D.E., Kammen, B.F., Lindpaintner, K., Bisbas, D. and Nadal-Ginard, B. (1993) Neuron, 10, 1121–1129.
[14] Smith-Maxwell, C.J., Kanevsky, M. and Aldrich, R.W. (1993) Biophys. J. 64, A200.
[15] Mulvey, D., King, G.F., Cooke, R.M., Doak, D.G., Harvey, T.S. and Campbell, I.D. (1989) FEBS Lett. 257, 113–117.
[16] Tosteson, M.T., Auld, D.S. and Tosteson, D.C. (1989) Proc. Natl. Acad. Sci. USA 86, 707–710.
[17] Haris, P.I. and Chapman, D. (1992) Trends Biochem. Sci. 17, 328–333.
[18] Merrifield, R.B. (1963) J. Am. Chem. Soc. 85, 2149–2154.
[19] Carpino, L.A. and Han, G.Y. (1972) J. Org. Chem. 37, 3404–3409.
[20] Haris, P.I., Lee, D.C. and Chapman, D. (1986) Biochim. Biophys. Acta 874, 255–265.
[21] Haris, P.I., Ramesh, B., Sansom, M.S.P., Kerr, I.D., Srai, K.S. and Chapman, D. (1993) Protein Engineering 7, 255–262.
[22] Guy, H.R. and Seetharamulu, P. (1986) Proc. Natl. Acad. Sci. USA 83, 508–512.
[23] Guy, H.R. and Conti, F. (1990) Trends Neurosci. 13, 201–206.
[24] Durrel, S.R. and Guy, H.R. (1992) Biophys. J. 62, 238–250.
[25] Leuchtag, H.R. (1994) Biophys J. 66, 217–224.
[26] Pusch, M. (1990) Eur. Biopys. J. 18, 327–333.
[27] Schwartz, A., Palti, Y. and Meiri, H. (1990) J. Membr. Biol. 116, 117–128.