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Rapid report

Polarity-dependent conformational switching of a peptide mimicking the S4-S5 linker of the voltage-sensitive sodium channel

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Abstract

The S4-S5 linker (or S45) in voltage-sensitive sodium channels was previously shown to be involved in the permeation pathway. The secondary structure, investigated by circular dichroism, of a S4-S45 peptide from domain IV and its fragments (including S45) is reported here and compared with that of the homologous peptide from domain II as a function of the solvent dielectric constant. The reduction in helicity seen for S4-S45 (II) in polar media is cancelled in membrane-like environment. The most striking result – a sharp α-helix $\rightarrow$ β-sheet transition upon exposure of the S45 moiety to aqueous solvents – is discussed as regards channel activation and selectivity.

Keywords: Sodium ion channel; Model peptide; Conformation; Circular dichroism

Cellular excitability is mediated through voltage-gated ionic channels, like the sodium channel, an integral membrane protein which fulfills a fundamental role in the generation and propagation of action potentials in nerve and muscle fibers. Sequences of the sodium channel protein have become available for several species and tissues [1–7]. In each case, the sodium channel molecule is a large (over 1800 residues) single-chain polypeptide organized into four homologous domains, each of which is composed of six predicted transmembrane segments and a amphipathic segment within the loop connecting transmembrane segments S5 and S6 (the P-region). Site-directed mutagenesis and electrophysiological studies of cDNA-injected oocytes (for a short review, see [8]) underline the essential roles of the S4 segments (helices with basic residues every three positions) as the main voltage-sensors [9], and of the P-regions as the selectivity filter [10]. These features are incorporated into topological and dynamical models [11,12] but the identification of 'physical or molecular gates' remain elusive.

Independent investigations employing site-directed mutagenesis of the whole channel on one hand [13] and the synthetic peptide approach followed by reconstitution into planar lipid bilayers [14] on the other hand recently confirmed the implication in the permeation pathway and ion selectivity of the short loop (S45) connecting on the inner side of the membrane the heavily-charged S4 and the highly hydrophobic S5 in each domain. Structural data concerning these channels being more than scanty, it seems appropriate to pursue the peptide strategy taking into account recent studies showing that various synthetic segments derived from membrane proteins adopt conformations similar to those of the relevant segments within the intact protein [15,16].

Here, employing circular dichroism (CD), we examine the effects of the solvent dielectric constant on the conformational contents and the stabilization of the secondary structure of analogous synthetic segments, namely S4-S45 of domain IV from *Electrophorus electricus* sodium channel and selected fragments of increasing lengths, and S4-S45 of domain II (see the sequences in Table 1). The results, especially the helix–β-sheet transition of S45 driven by changes in the solvent polarity are discussed as regards their eventual implication for channel activation and selectivity.

Materials. Boc-amino acids were obtained from Bachem (Bubendorf, Switzerland). Other reagents for peptide synthesis included trifluoroacetic acid (TFA, from Sigma),

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N,*N*-diisopropylethylamine (DIEA, from Aldrich), dicyclohexylcarbodiimide (DCC, from Fluka), 1-hydroxybenzotriazole (Fluka), methylene chloride (Carlo Erba), and dimethyl formamide (DMF, from Aldrich). HPLC grade acetonitrile was from Janssen. 2,2,2-Trifluoroethanol (TFE) for synthesis grade was purchased from Merck (Darmstadt, Germany) and 2-propanol HPLC grade from Carlo Erba (Milan, Italy).

Peptide synthesis and purification. The S4-S45 and S45 peptides of fourth domain were prepared by the solid-phase technique [17] on a SAP 4 synthesizer model from Sempa-Chimie (Paris). The *t*-BOC strategy was used on a benzhydrylamine resin reticulated by 1% divinylbenzene. To avoid oxidization hazards during the subsequent purification, the two Met residues near the C-terminus were replaced by *nor*-Leu. After I24, R16 and L8 had been coupled (see Table 1), aliquots of the peptidyl-resin were put aside before completion of the synthesis. The peptide and its fragments, the first of which (11 residues) corresponding to S45, were then released from the resin and side chain protecting groups eliminated by the usual fluorohydric acid treatment. The S4-S45(II) was prepared by the Fmoc strategy on a 433.A Applied Biosystem. The lyophilized raw products were purified on a Nucleosil C_{18} reverse phase semi-preparative column (10 μm particle, 8 × 300 mm), from Société Française Chromato Colonne/Shandon Scientific (Eragny, France) at a flow rate of 1.5 ml/min, under acetonitrile/H_2O gradients with 0.1% TFA. Peptide homogeneity was determined by analytical HPLC using an acetonitrile gradient with 0.1% TFA and the molecular mass and amino acid sequence were checked by electrospray-ionization and FAB (fast atomic bombardment) mass spectrometry, respectively.

Circular dichroism (CD) spectroscopy. CD spectra were recorded from peptide solutions (1 mg/ml) in H_2O/TFE or 2-propanol (in varying proportion) using a Dichrograph Mark V spectropolarimeter (Longjumeau, France) at room temperature in steps of 1 nm. The instrument had previously been calibrated with the ellipticity values and peaks of a standard solution of *d*-10-camphorsulfonic acid. All ellipticity measurements are reported as mean residue ellipticity, $[\theta]$, having the units deg cm^2 dmol^{-1}. Each spectrum was the average of five repeated scans. The sensitivity was set at either $5 \cdot 10^{-6}$ or $1 \cdot 10^{-5}$, the path length at 0.01 cm and the time response at 5 s in the range of 185–260 nm. The different conformational contents were estimated from 51 mean residue ellipticity values (from 190 to 240 nm, every nanometer) and computed using the standards of Chang et al. [18] except for the short loop S45 where the use of Bolotina et al.'s reference spectra (from 200 to 250 nm, every nanometer) [19] yields a better fit as judged by the normalized root mean square (NRMS) deviations [20].

Table 1
Amino acid sequences of the peptides and fragments studied

S4-S45(IV) and fragments:

Ac-TLFRVIR LARIARVL RLIRAAKG IRTLLFALMMS-NH2

27-mer ←——————————→

19-mer ←——————→

11-mer ←——S45——→

S4-S45(II):

Ac-SVLRSLRLLRIFKLAKSWPTLNILIKI ICNSVGA-NH2

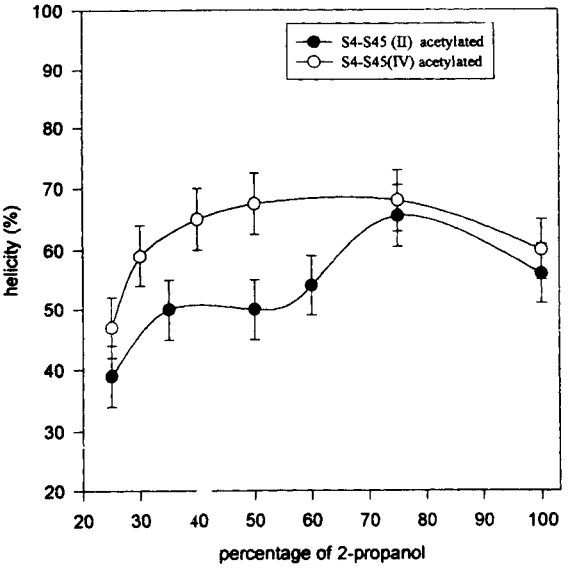

Fig. 1. Compared helical contents for S4-S45 peptides (1 mg/ml) of domains IV and II (influence of proline) as a function of 2-propanol percentage.

Results and discussion. Fig. 1 compares the helicities of S4-S45 peptides of the second and fourth domains as a function of 2-propanol percentage in H_2O/2-propanol solvent. Increasing 2-propanol proportion stabilizes the helical conformation (up to 60–70%) for both segments, low polarity media favoring intra-molecular hydrogen bonding. This occurs at the expense of mainly the unordered conformation, confirming previous findings using Fourier Transform Infra-Red spectroscopy [21]. Below 70% 2-propanol, the significantly reduced S4-S45(II) helicity, as compared to corresponding values for the homologous peptide from domain IV, is consistent with the classical effect of the proline residue (here at position 19 in S4-S45 from domain II), i.e. helix kinking which leaves an unordered structure for the rest of the molecule. This was also observed in a 2D-NMR study on the S4 segment of the first domain of rat brain I and II sodium channels [22]. Nevertheless and quite surprisingly, a S-shaped evolution is observed from 50% to 65% helicity when 2-propanol is increased from 50% to 75% (Fig. 1). Thus, in spite of this potential helix kinker (proline), the conformations of both peptides – and

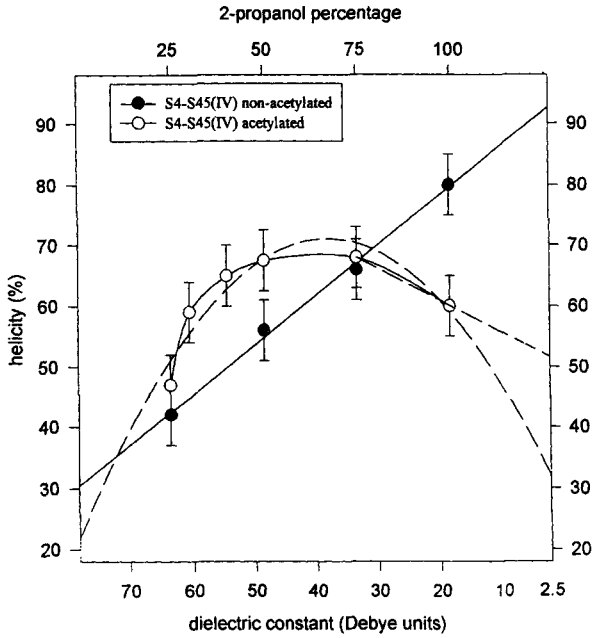

Fig. 2. Helicity as a function of solvent dielectric constant (aqueous solution of 2-propanol at 1 mg peptide/ml): influence of acetylation of S4-S45(IV).

this could also be inferred for domains I and III whose S4 possess a proline – is quite similar in media mimicking the membrane environment and possibly in the channel itself. This may explain the lack of any detectable effect on the steepness of activation curves upon mutating these Pro residues in electrophysiological experiments [23].

In Fig. 2, S4-S45(IV) helicity is plotted as a function of the solvent dielectric constant, with

$$\varepsilon = \varepsilon_{H_2O} \cdot \%[H_2O] + \varepsilon_{2\text{-propanol}} \cdot \%[2\text{-propanol}]$$

($\varepsilon_{2\text{-propanol}}$ being 18.3 Debye units). When ε decreases (high 2-propanol content), the helicity of the acetylated S4-S45(IV) segment reaches a plateau (around 70%) before decreasing again for very high 2-propanol percentage. Extrapolating back to the dielectric constant typical for the hydrophobic core of the lipid bilayer ($\varepsilon = 2.5$), the range of helicity values (33–52%) matches those we previously found when using zwitterionic or negatively-charged lipid vesicles (SUV), respectively, 37% and 54% [14]. The helicity of the non-acetylated analogue displays a linear dielectric constant-dependence and reaches 80%–90% in low polarity media. Here, the C-terminal charge and its shielding seems predominant.

The conformation of the S4-S5 loop (S45 peptide) exhibits a major transition between 25% and 20% TFE yielding more than 90% β-sheet conformation from random and helix conformations, as shown from the family of CD spectra (Fig. 3A) and their analysis (Fig. 3B). It should be mentioned here that both solvents (TFE and 2-propanol) yielded similar results. As seen from Table 2 which compares helical and β-sheet contents of S4-S45(IV) and its overlapping fragments, the number of S45 residues in-

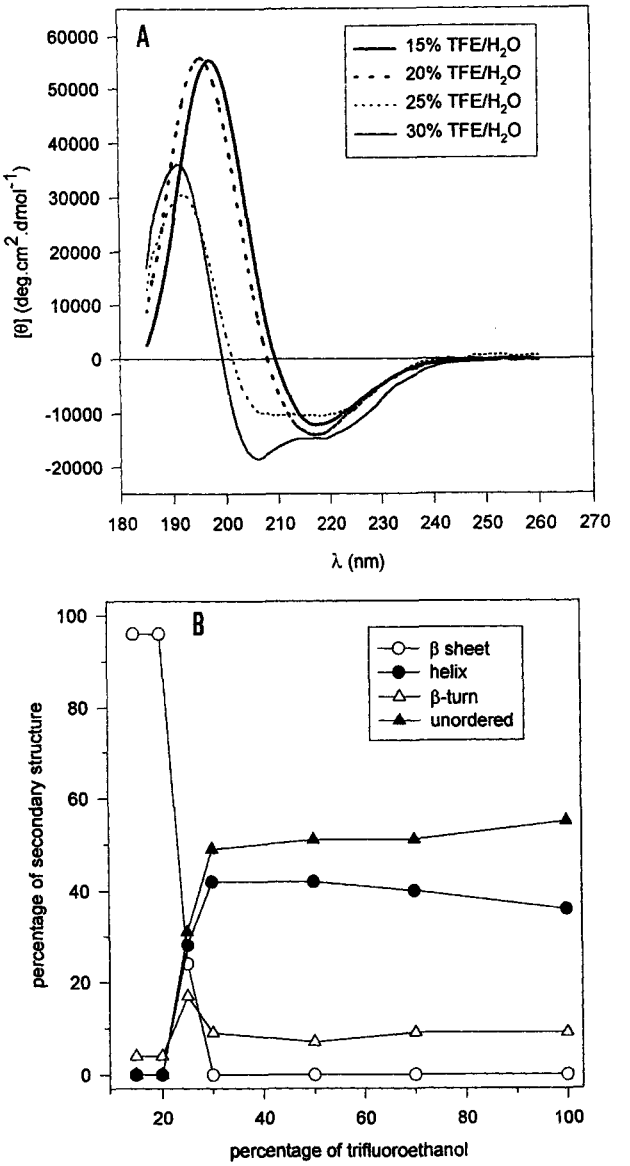

Fig. 3. (A) CD spectra of the S45 linker in TFE/H_2O solutions at 1 mg/ml and room temperature (25°C). (B) Analysis of CD spectra shown in (A) illustrating a sharp (helix + unordered) → β-sheet transition upon exposure to aqueous solvents.

Table 2
Helical and β-sheet contents of S4-S45 and its fragments of fourth domain (down to S45) in media of high and low dielectric constant

Peptide length (number of residues)	15% TFE		75% TFE	
	helix %	β-sheet %	helix %	β-sheet %
11 (S45)	0 (0)	96 (11)	40 (4)	0 (0)
19	38 (7)	17 (3)	60 (11)	0 (0)
27	45 (12)	16 (4)	63 (17)	0 (0)
34 (S4-S45)	47 (16)	17 (6)	68 (23)	2 (1)

In parentheses are indicated the number of residues in helix or sheet.

volved in this conformational change is reduced from 11–10 to 6 when this moiety is attached to the S4 segment. For nonpolar environment mimicking membranes, the helical content increase with the length of peptides allows to infer a major helix in the whole peptide. CD results (Table 2) would suggest a split into one major helix corresponding to S4 segment, in agreement with the NMR study of a analogous peptide [22] and one minor in the C-terminal part as revealed by helicity of S45 peptide alone and its two longer overlapping segments. In the high polarity medium (15% TFE), helicity of the whole S4-S45(IV) is reduced from 68% to 47% arguing for a 16-residue helix. Thus, the S4 core would be relatively stabilized in the helical conformation and the helix → coil transition [21] driven by exposure to aqueous media would be more restricted than postulated by theoretical suggestions involving the whole S4 segment [24]. On the other hand, our results are compatible with the recognition of a large portion of S4 segments by monoclonal antibodies upon channel activation [25], helices favoring such recognition.

Although some caution is needed when discussing the eventual effect of the polarity-dependent conformational switching of the S45 peptide in isolation on the gating of the intact channel, the effect reported here, even limited to 6 residues in the whole S4-S45, could suggest that the inwardly-directed β-sheet promotion, concomitant with the outwardly-directed 'propagating helix' both previously postulated for S4 during channel activation [11], may well extend to the S45 moiety. This would also account for the 'flexible or spring' region at the junction of S4 and S45 in the context of Durell and Guy's model [12]: S45 would only be drawn inside the pore lumen once S4 had been largely exposed to the extracellular side. This short S45 segment moving from a membrane surrounding to an aqueous environment as in our CD experiments could undergo some β-sheet transition. We then propose that the intracellular part of the activated pore could then be made of a β-barrel (the four S45s, 6 residues in β-sheet conformation encompassing half the bilayer width) in series with the main selectivity filter, presumably made up by the anti-parallel arrangement of the short SS1 and SS2 segments (the P-region). Finally, it is noteworthy to mention in this context that a recent study [26] demonstrates the adoption of a β structure by the inactivating 'ball peptide' whose receptor is assumed to be the S45 region.

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