

# Solution structure and membrane-binding property of the N-terminal tail domain of human annexin I

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**Abstract** The conformational preferences of AnxI<sup>N26</sup>, a peptide corresponding to residues 2–26 of human annexin I, were investigated using CD and NMR spectroscopy. CD results showed that AnxI<sup>N26</sup> adopts a mainly  $\alpha$ -helical conformation in membrane-mimetic environments, TFE/water and SDS micelles, while a predominantly random structure with slight helical propensity in aqueous buffer. The helical region of AnxI<sup>N26</sup> showed a nearly identical conformation between in TFE/water and in SDS micelles, except for the orientation of the Trp-12 side-chain, which was quite different between the two. The N-terminal region of the AnxI<sup>N26</sup> helix showed a typical amphipathic nature, which could be stabilized by the neighboring hydrophobic cluster. The helical stability of the peptide in SDS micelles was increased by addition of calcium ions. These results suggest that the N-terminal tail domain of human annexin I interacts with biological membranes in a partially calcium-dependent manner. © 2000 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

**Key words:** AnxI<sup>N26</sup>; Human annexin I; Nuclear magnetic resonance; Circular dichroism; Membrane binding

## 1. Introduction

Annexins are a family of ubiquitous proteins that bind to negatively charged phospholipids in a calcium-dependent manner, and are known to be involved in many important cellular processes such as anti-inflammation, anti-coagulation, membrane trafficking, etc. [1,2]. Each annexin is composed of two parts, a major C-terminal core domain and a minor N-terminal tail domain. The similar properties of all annexins regarding Ca<sup>2+</sup> and phospholipid seem to be due to the C-terminal core domains that show highly conserved sequences and structures. Since the N-terminal tail domains of annexins differ widely in length and sequence, it has been proposed that the structurally distinct N-terminal domain of each annexin may impart functional specificity [3–7]. Indeed, there have

been many experimental reports on the different functional roles of the N-terminal tail domains of annexins [4–9].

Although the 3D structures of more than 10 annexins are presently available [8–17], our knowledge of the N-terminal domain structure of annexins is very scant because these N-terminal domains are either truncated, as in the case of annexin I, or naturally short. Particularly, no structural evidence has been reported for whether the N-terminal tail of annexin I itself binds to membrane, although many reports have noted that the N-terminal region of annexin I regulates its membrane interaction properties [18–22].

The N-terminal tail domain of annexin I consists of 41 amino acids. However, annexin I lacking residues 1–26 frequently exists in vivo [23]. It is known that the residue Lys-26 is prone to tryptic digestion, and the membrane binding of the annexin I core domain is regulated by the truncation of the tail domain at this position [20–22]. These facts suggest that residues 1–26 of annexin I could interact with the membrane. In addition, annexin I has a unique tryptophan at position 12 in the N-terminal tail domain. Tryptophan residues are frequently found at the lipid bilayer interface in membrane proteins, where they may be involved in stabilizing correct protein–membrane interactions [1,24,25]. In particular, the unique tryptophan of annexin V plays a crucial role in its membrane binding [26,27].

In this paper, we report the conformational preferences of the partial N-terminal domain of annexin I at membrane-mimetic environments by using a peptide corresponding to residues 2–26 of human annexin I (AnxI<sup>N26</sup>). The results of the present report provide structural evidence for the membrane-binding properties of AnxI<sup>N26</sup>, including the role of the hydrophobic cluster containing the unique tryptophan of annexin I. Additionally, the effect of Ca<sup>2+</sup> on the membrane-binding property of the peptide is investigated.

## 2. Materials and methods

### 2.1. Materials and peptide preparation

The synthetic peptide N-acetyl-AMVSEFLKQAWFIENEEQEYVQTVK-COOH, corresponding to residues 2–26 of human annexin I, was purchased from Chiron, Australia. The sequence and purity of the peptide were confirmed by mass-spectroscopy and high performance liquid chromatography. 2,2,2-trifluoroethanol-*d*<sub>3</sub> 99.5% (TFE-*d*<sub>3</sub>) and sodium 4,4-dimethyl-4-silapentane-1-sulfonate (sodium dodecyl sulfate (SDS)-*d*<sub>25</sub>) were obtained from Aldrich. D<sub>2</sub>O 99.95% was obtained from Sigma, and all other chemicals were analytical grade obtained from various manufacturers.

### 2.2. Circular dichroism (CD) spectroscopy

CD spectra were obtained at 20°C on a JASCO J-715 spectropo-

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**Abbreviations:** AnxI<sup>N26</sup>, a peptide corresponding to residues 2 to 26 of human annexin I; CD, circular dichroism; DQF-COSY, double quantum filtered correlation spectroscopy; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; r.m.s.d., root mean square deviation; SDS, sodium dodecyl sulfate; TFE, 2,2,2-trifluoroethanol; TOCSY, total correlation spectroscopy

larimeter, using a 1 mm path-length cell, with a 1 nm bandwidth, 0.5 nm step resolution, 4 s response time, and a scan speed of 20 nm min<sup>-1</sup>. Four scans were added and averaged, followed by subtraction of the CD signal of the solvent. Samples were prepared by dissolving the peptide to the concentration of 0.1 mg ml<sup>-1</sup> in various solvents: 50 mM potassium phosphate buffer (pH 6.5), TFE/water mixtures, and 10 mM SDS. Calcium ions were added by a stock solution of 150 mM CaCl<sub>2</sub>.

### 2.3. Nuclear magnetic resonance (NMR) spectroscopy

Samples for NMR measurements contained 3 mM peptide in TFE-*d*<sub>3</sub>/H<sub>2</sub>O (1:1, v/v) at pH 4.0, and in 300 mM SDS-*d*<sub>25</sub> at pH 4.0. All NMR spectra were recorded at 40°C by using a Bruker DRX-500 spectrometer equipped with a gradient unit. The WATERGATE sequence [28] was used to suppress the solvent signals in the nuclear Overhauser effect spectroscopy (NOESY) and total correlation spectroscopy (TOCSY) experiments. For the double quantum-filtered correlation spectroscopy (DQF-COSY) experiments, solvent suppression was achieved using selective low-power irradiation of the water resonance. The 2D TOCSY spectra were acquired with isotropic mixing times of 40 and 60 ms, respectively. The 2D NOESY spectra were acquired with mixing times of 200 and 300 ms, respectively. Slowly exchanging amide protons were monitored with a series of 2D NOESY spectra recorded at 5, 50, 140, 230, 320, 410 and 500 min on AnxI<sup>N26</sup> solutions in TFE-*d*<sub>3</sub>/D<sub>2</sub>O (1:1) and in 300 mM SDS-*d*<sub>25</sub>/D<sub>2</sub>O, respectively. All NMR spectra were processed and analyzed using the NMRPipe/NMRDraw software and the NMRView program [29,30]. Chemical shifts were referenced to methyl signals of sodium 4,4-dimethyl-4-silapentane-1-sulfonate.

### 2.4. Structure calculation

Distance restraints were obtained from the NOESY spectra. NOE data from the NOESY spectra were classified into three classes; strong, medium, and weak, corresponding to upper bound interproton distance restraints of 3.0, 4.0, and 5.0 Å, respectively. Lower distance bounds were taken as the sum of the van der Waals radii of 1.8 Å. Pseudoatom corrections were applied to non-specifically assigned methylene, methyl, and aromatic ring protons [31]. Hydrogen bond restraints were determined on the basis of slowly exchanging amide protons and the pattern of the NOEs characteristic of an  $\alpha$ -helix. The 3D structures were calculated using the simulated annealing and energy minimization protocol in the program XPLOR 3.851 [32].

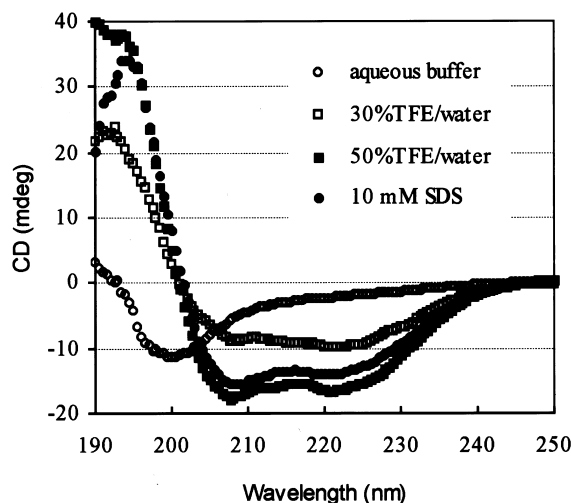


Fig. 1. CD spectra of AnxI<sup>N26</sup> in 50 mM potassium phosphate buffer (○), in 30% TFE/water mixture (□), in 50% TFE/water mixture (■), and in 10 mM SDS (●).

## 3. Results and discussion

### 3.1. Conformational preferences of AnxI<sup>N26</sup>

The conformational preferences of AnxI<sup>N26</sup> at different solvent environments were investigated by CD spectroscopy (Fig. 1). In aqueous buffer, the CD spectrum of AnxI<sup>N26</sup> showed a strong negative band at 200 nm and a broad band around 222 nm, indicating a predominantly random-coil conformation with a slight helical propensity [33,34]. However, in TFE/water mixtures, the CD spectra showed double minima at 208 and 222 nm, an isodichroic point at 200 nm, and a positive band at 193 nm, which are indicative of a highly  $\alpha$ -helical conformation [35–37]. The signals at 193, 208, and 222 nm were intensified with increasing concentrations of TFE, which

Table 1

<sup>1</sup>H chemical shifts of AnxI<sup>N26</sup> in 50% TFE and in 300 mM SDS micelles, at pH 4.0 and 40°C

	50% TFE			Others	300 mM SDS			Others
	H <sup>N</sup>	H <sup>α</sup>	H <sup>β</sup>		H <sup>N</sup>	H <sup>α</sup>	H <sup>β</sup>	
A2	8.04	4.24	1.48		8.30	4.20	1.48	
M3	8.21	4.44	2.14	n.d. <sup>a</sup>	8.42	4.39	2.12/2.24	H <sup>γ</sup> 2.68/2.56
V4	7.56	3.92	2.23	H <sup>γ</sup> 1.07/1.10	7.80	3.83	2.27	H <sup>γ</sup> 1.10/1.04
S5	7.92	4.22	4.03		8.07	4.11	3.99	
E6	7.86	4.23	2.24	H <sup>γ</sup> 2.55	7.84	4.15	2.21/2.14	H <sup>γ</sup> 2.50
F7	8.00	4.43	3.32	H <sup>δ/ε</sup> 7.28/7.26	7.99	4.40	3.26	H <sup>δ/ε/ζ</sup> 7.24/7.22/7.26
L8	8.27	4.09	1.92	H <sup>γ</sup> 1.51, H <sup>δ</sup> 1.07/0.97	8.21	4.00	1.99/1.94	H <sup>γ</sup> 1.52, δ 0.95
K9	7.86	4.15	2.02	H <sup>γ</sup> 1.73/1.53, H <sup>δ</sup> 1.50	7.92	4.09	1.95	H <sup>γ/δ</sup> 1.52/1.70
Q10	7.95	4.28	2.35/2.18	H <sup>γ</sup> 2.59/2.46, H <sup>ε</sup> 6.69/5.33	7.80	4.40	2.16	H <sup>γ</sup> 2.50
A11	8.30	4.09	1.44		8.14	4.04	1.30	
W12	8.38	4.34	3.45/3.54	H <sup>δ/ε/ζ</sup> 7.17/7.54/7.56	8.25	4.39	3.40	H <sup>δ/ε/ζ</sup> 7.24/7.50/7.51
F13	8.30	4.08	3.38	H <sup>δ/ε</sup> 7.35/7.34	7.70	4.12	3.14/3.06	H <sup>δ</sup> 7.17/7.16
I14	8.40	3.70	2.05	H <sup>γ1</sup> 1.94/1.33, H <sup>γ2</sup> 0.97, H <sup>δ</sup> 0.94	7.84	3.71	2.01	H <sup>γ1</sup> 1.71/1.29, H <sup>γ2</sup> 0.90
E15	8.45	4.12	2.28	H <sup>γ</sup> 2.61/2.47	8.23	3.96	2.14/2.03	H <sup>γ</sup> 2.45
N16	8.21	4.03	2.08	H <sup>δ</sup> 7.13/6.49	8.05	4.28	2.46	H <sup>δ</sup> 7.09/6.03
E17	8.21	4.44	2.14	H <sup>γ</sup> 2.57/2.67	7.91	4.14	2.05	H <sup>γ</sup> 2.50
E18	8.03	4.10	2.20	H <sup>γ</sup> 2.45/2.55	8.26	4.18	2.04	H <sup>γ</sup> 2.35/2.50
Q19	8.45	4.06	2.20	H <sup>γ</sup> 2.71	8.06	4.03	2.11	H <sup>γ</sup> 2.45
E20	7.98	4.12	2.20	H <sup>γ</sup> 2.56	7.91	4.14	2.03	H <sup>γ</sup> 2.50
Y21	8.21	4.29	3.27/3.19	H <sup>δ/ε</sup> 7.12/6.80	7.98	4.39	3.15/3.03	H <sup>δ/ε</sup> 7.10/6.79
V22	8.23	3.76	2.26	H <sup>γ</sup> 1.06/1.18	8.12	3.84	2.22	H <sup>γ</sup> 1.08/1.00
Q23	7.87	4.23	n.d.	n.d.	7.91	4.26	2.10/2.17	H <sup>γ</sup> 2.43, H <sup>ε</sup> 7.37/6.72
T24	7.79	4.30	4.30	H <sup>γ</sup> 1.29	7.77	4.31	4.26	H <sup>γ</sup> 1.25
V25	7.76	4.07	2.03	H <sup>γ</sup> 0.85/0.89	7.70	4.12	2.09	H <sup>γ</sup> 0.92
K26	7.88	4.37	1.91	H <sup>γ/δ/ε</sup> 1.49/1.72/3.04	7.81	4.29	n.d.	H <sup>γ</sup> 1.46

<sup>a</sup>n.d., not detected.

indicates that the helicity of the peptide increased at more hydrophobic environments. The CD spectrum of AnxI<sup>N26</sup> in 10 mM SDS, which is above the critical micellar concentration of SDS [38], also showed a typical  $\alpha$ -helix pattern. This conformational behavior of AnxI<sup>N26</sup> is common to many membrane-binding peptides [33–37,39–41]. Thus, it can be suggested that the conformational transition of AnxI<sup>N26</sup>, from random-coil in aqueous buffer to  $\alpha$ -helix in membrane-mimetic environments, reflects its binding to the membrane.

### 3.2. NMR structures of AnxI<sup>N26</sup>

The helical structures of AnxI<sup>N26</sup> in TFE/water and in SDS micelles were precisely investigated by NMR spectroscopy. Sequence-specific assignments of the proton resonances were achieved by spin system identification from TOCSY and DQF-COSY spectra, followed by sequential assignments through NOE connectivities [31]. Table 1 lists the nearly complete assignments of the proton chemical shifts of AnxI<sup>N26</sup> in 50% TFE/water and in 300 mM SDS micelles. As shown in Fig. 2, the presence of a typical  $\alpha$ -helix from V4 to Q19 is supported, both in TFE/water and in SDS micelles, by the frequent observations of  $d_{NN}(i,i+1)$ ,  $d_{NN}(i,i+2)$ ,  $d_{\alpha N}(i,i+3)$ ,  $d_{\alpha N}(i,i+4)$  NOE connectivities, and slowly exchanging amide protons. A set of 50 structures of AnxI<sup>N26</sup> in 50% TFE/water was calculated by using 268 distance restraints (105 intraresidue, 98 sequential, and 65 medium range restraints) and 28 hydrogen bond restraints. Out of the 50 structures, 49 structures without distance violation larger than 0.5 Å were accepted, and 20 structures with the lowest energies finally selected through several rounds of refinements. In the same manner, the final 20 structures of AnxI<sup>N26</sup> in SDS micelles were selected by using 240 distance restraints (80 intraresidue, 94 sequential, and 66 medium range restraints) and 26 hydrogen bond restraints. The structural statistics for the 20 final structures of AnxI<sup>N26</sup> in TFE/water and in SDS micelles are summarized in Table 2. Fig. 3 shows superimpositions of the backbone atoms of the finally selected AnxI<sup>N26</sup> structures in TFE/water and in SDS micelles, respectively. The overall structure of AnxI<sup>N26</sup> in SDS micelles is similar to that in TFE/water, and particularly the conformation of the helical part (V4–Q19) is nearly identical. The relatively disordered conformation in the C-terminal region of AnxI<sup>N26</sup> may be attributed, both in TFE/water and in SDS micelles, to the

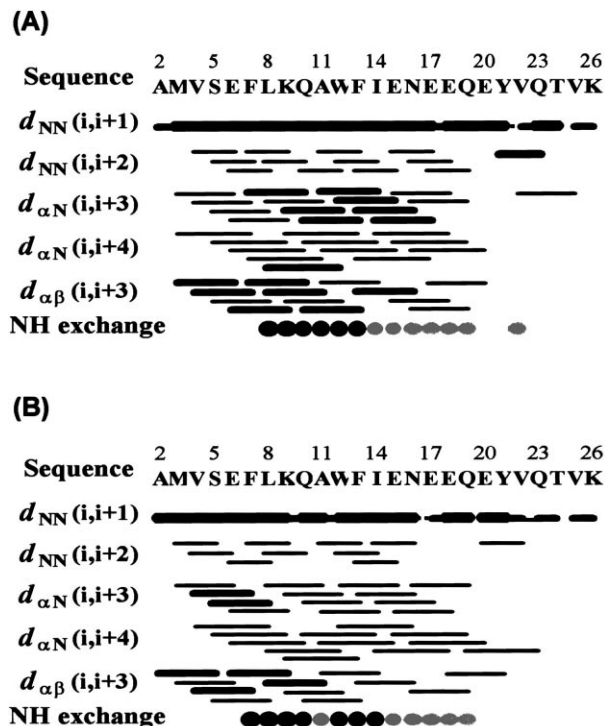


Fig. 2. Overview of the NOE connectivities and amide proton exchange rates of AnxI<sup>N26</sup> in TFE/water mixture (A) and in SDS micelles (B). Moderately and slowly exchanging amide protons are represented as gray circles and filled circles, respectively.

break of the peptide bond between AnxI<sup>N26</sup> and the core of annexin I.

### 3.3. Structural characteristics for membrane binding

The insight into the spatial structure of AnxI<sup>N26</sup> shows three structural advantages of the peptide for binding to membrane. To begin with, the N-terminal part (V4–W12) of the AnxI<sup>N26</sup> helix (V4–Q19) shows a typical amphipathic character, which is depicted in Fig. 4A. In many cases, the amphipathic nature of a helical peptide is known to be important for membrane binding [33–36,39]. It seems that the non-polar side chains that extend from the hydrophobic face of AnxI<sup>N26</sup> could interact with the hydrophobic acyl chains of micelles, as proposed for other amphipathic peptides [36].

Table 2

Structural statistics for the 20 converged structures of AnxI<sup>N26</sup> in 50% TFE and in 300 mM SDS

Statistics for structure calculations	50% TFE	300 mM SDS
R.m.s.d. from idealized covalent geometry		
Bonds (Å)	0.0024 ± 0.0001	0.0011 ± 0.0001
Bond angles (°)	0.462 ± 0.006	0.441 ± 0.006
Improper torsion (°)	0.333 ± 0.006	0.315 ± 0.005
R.m.s.d. from experimental restraints		
Distances (Å)	0.021 ± 0.002	0.006 ± 0.004
Final energies (kcal mol <sup>-1</sup> )		
<i>E</i> <sub>total</sub>	39.2 ± 1.8	27.7 ± 1.3
<i>E</i> <sub>bond</sub>	2.4 ± 0.2	0.5 ± 0.1
<i>E</i> <sub>angles</sub>	24.8 ± 0.6	22.6 ± 0.6
<i>E</i> <sub>improper</sub>	3.8 ± 0.6	3.4 ± 0.1
<i>E</i> <sub>vdW</sub>	3.0 ± 0.6	0.7 ± 0.5
<i>E</i> <sub>NOE</sub>	5.1 ± 0.9	0.5 ± 0.5
Average r.m.s.d. to the mean structure for all atoms/the backbone (Å)		
Whole (2–26)	4.16/2.71	3.10/2.04
Helix (4–19)	1.07/0.30	1.27/0.42

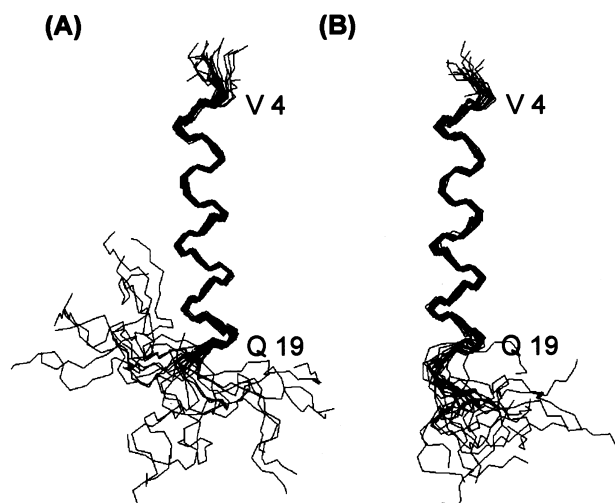


Fig. 3. Superimposition of the backbone atoms (N, C $\alpha$ , and C') of 20 structures in TFE/water mixture (A) and in SDS micelles (B). Residues 4–19 of AnxI<sup>N26</sup> were overlapped with the average r.m.s.d. values of 0.30 and 0.42 Å for the backbone, respectively.

Second, the hydrophobic cluster ranging from A11 to I14 (-A-W-F-I-) can stabilize the neighboring amphipathic part and play a key role in membrane binding. As shown in Fig. 4B, the aromatic rings of W12 and F13 of the refined average structure of AnxI<sup>N26</sup> are oriented more or less perpendicular to each other, which is probably due to a favorable interaction between the two rings [42]. Such aromatic–aromatic interactions have been found to stabilize protein structures, and particularly a hydrophobic cluster including the WF pair is important for the stabilization of the N-terminal amphipathic helix of human apolipoprotein E [42,43].

Finally, the residue W12, which is the unique tryptophan residue of annexin I, seems to be crucial for the membrane interaction of AnxI<sup>N26</sup>. When the helical part (V4–Q19, 269 atoms) of the refined average structure of AnxI<sup>N26</sup> in SDS micelles was overlaid with that in TFE/water, the two structures were matched well with the root mean square deviation (r.m.s.d.) value of 0.22 Å for the backbone (Fig. 4B). How-

ever, the orientation of the W12 side chain was remarkably different between the two. Since the SDS micelle is heterogeneous, it is a more membrane-mimetic environment than TFE. Thus, the different orientation of the W12 side chain seems to reflect the anchoring role of the residue in the membrane-binding process of AnxI<sup>N26</sup>, as in the cases of other membrane-binding proteins [24–27].

### 3.4. Effect of calcium on AnxI<sup>N26</sup>

It has been proposed that calcium binding may alter the conformation of the N-terminal region of annexin I, and reciprocally, that alterations in its N-terminal region can modify its calcium-binding properties [6,19–20]. Thus, we investigated the calcium-binding ability of AnxI<sup>N26</sup> in SDS micelles by CD spectroscopy (Fig. 5). By an addition of calcium, the CD signals of AnxI<sup>N26</sup> in SDS micelles were intensified at 193, 208, and 222 nm, which indicates that the helical structure of the peptide was more stabilized. No further spectral change was observed with above 5 equimolar calcium, which indicates a saturation. The calcium-dependent peripheral binding of annexins to membrane is generally assumed to function via a 'Ca<sup>2+</sup>-bridge', in which the Ca<sup>2+</sup>-ion is coordinated by polar headgroups of the phospholipid molecules and by ligands from the proteins [18,44–45]. In annexins, the acidic side-chains of glutamate residues often contribute to the formation of a Ca<sup>2+</sup>-binding site [1,45]. The C-terminal region of the AnxI<sup>N26</sup> helix contains frequent glutamate residues (-E15-N-E-E-Q-E20-; Fig. 4A). Thus, we suggest that Ca<sup>2+</sup>-ions bind to the negatively charged glutamate-rich region of AnxI<sup>N26</sup> and function as salt-bridges between the peptide and SDS micelle molecules. The calcium-bridge in this region seems to stabilize the helical conformation of AnxI<sup>N26</sup> as a membrane-bound form, resulting in the intensification of CD signals as shown in Fig. 5.

### 3.5. Concluding remarks

This report constitutes the first detailed structural data of the N-terminal tail domain of annexin I. Since we studied its corresponding peptide, the structure of the N-terminal tail domain of annexin I in its intact form is not clear yet. However, present results indicate that the annexin I N-terminal tail

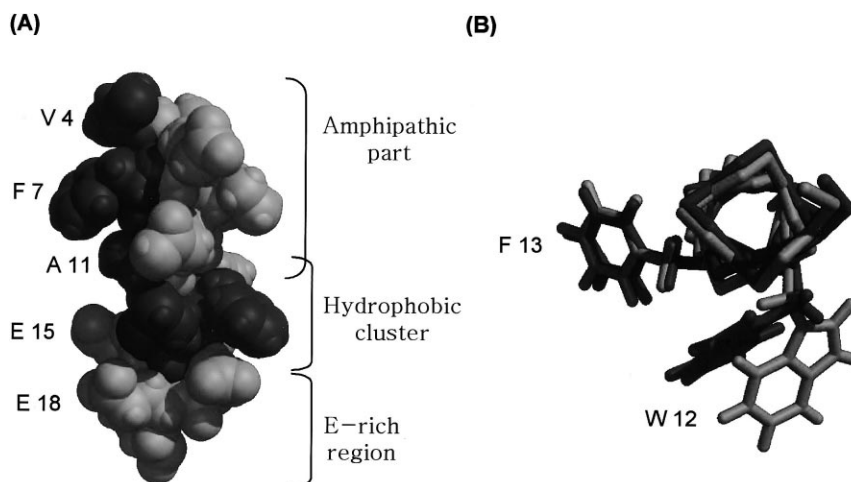


Fig. 4. A: Refined average structure of AnxI<sup>N26</sup> in SDS micelles. Residues 4–19 are shown as a conic presentation. Hydrophobic and hydrophilic residues are colored dark and light gray, respectively. B: Superposition of the refined average structures of AnxI<sup>N26</sup> in TFE/water (dark) and in SDS micelles (light). Backbone of residues 4–19 and side chains of the W12 and F13 residues are shown as a neon presentation. The direction of view is perpendicular (A) and parallel (B) to the helical axis, respectively.

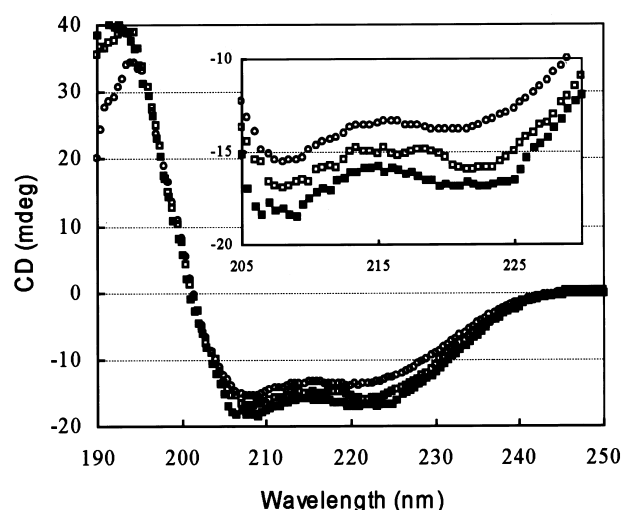


Fig. 5. CD spectra of AnxI<sup>N26</sup> in the absence of calcium (○), in the presence of three equimolar calcium (□), and in the presence of 5 equimolar calcium (■). All of the spectra were obtained with 0.1 mg ml<sup>-1</sup> of the peptide in 10 mM SDS micelles at 20°C. The region from 205 to 230 nm is enlarged in the inset.

itself probably binds to membrane even in the absence of calcium, forming a stable amphipathic helix. In addition, its membrane-bound state seems to be partially affected by calcium binding. A similar conformational behavior has been proposed for the annexin II N-terminal tail, for which the amphipathic  $\alpha$ -helical conformation is probably induced by its protein–protein interaction with p11 [7]. The N-terminal tail of annexin I, at least the first 12 amino acids, has been also proposed to interact with other proteins [4,5]. Particularly, it was confirmed recently that not only annexin I but also its N-terminal peptide, which corresponds to AnxI<sup>N26</sup>, has antiinflammatory effects by serving as a ligand for the formyl peptide receptor on human neutrophils [5]. Accordingly, the structure formation of the N-terminal domain of annexin I in the state of protein–protein contact with other proteins as well as the core of the protein itself might also be induced and would be worthy of investigation.

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