FEBS Letters 350 (1994) 169–172

FEBS 14311

Computer analyses suggest interactions of non-muscle filamin with lipid membranes

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Received 19 May 1994

Abstract

It is concluded from structure predictions of the primary amino acid sequence by computer analyses that two segments of non-muscle filamin could facilitate lipid membrane attachment or anchoring. Residues 49–71 of the amino-terminal may attach to phospholipid membranes, and residues 131–155 may anchor in the hydrophobic region of lipid membranes.

Key words: Filamin-lipid interaction; Sequence analysis; Structure prediction

1. Introduction

Filamin is a major constituent of the microfilament network determining the three-dimensional arrangement of actin filaments in smooth muscle and non-muscle cells [1]. Besides binding of filamin to actin [2], filamin–lipid interactions were proposed [3–5], suggesting a possible cytoskeletal–membrane linkage. In this work computational methods were applied in the search (within the sequence of ABP-280; non-muscle filamin; total residues 2647) for segments which might facilitate a hydrophobic and/or amphipathic attachment of the protein to phospholipid membranes.

2. Materials and methods

The primary amino acid sequence of human endothelial cell filamin (non-muscle filamin, ABP-280) was used for structure predictions by computer simulations [6]. The secondary structure was analyzed according to the SIMPA method [7]. SIMPA searches for local sequence homologies, using an internal data base of non-homologous polypeptide chains, and predicts alpha-helices, beta-sheets and random-coils. Beta-turns were predicted, applying the methods of Chou and Fasman [8]. The critical value for the prediction of a beta-turn was set to $> 10^{-4}$ [9]. Using the Genetic Computer Group program package, the calculations were carried out via the programs PREDICT (for SIMPA) and PEPTIDSTRUCTURE (for beta-turns).

In order to search for highly hydrophobic or amphipathic segments within the ABP-280 amino acid sequence, plots for the average hydrophobicity [10] and the average hydrophobic moment [11] were constructed. The normalized 'consensus' scale of Eisenberg et al. [11] was taken as the hydrophobicity scale for the amino acids. An amino acid window size of 19 was used, and the results were plotted above the middle residue of the window. The hydrophobic moment of alpha-helices and beta-strands was calculated, assuming a periodicity in the hydrophobicity of 3.6 residues and 2.0 residues, respectively.

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3. Results and discussion

According to the computer analysis of Gorlin et al. [6] about 90% of the sequence of ABP-280 is made up of 24 repeats of planar cross-beta-sheet configuration. The repeats with an average length of 96 residues begin at residue 276 and continue to the carboxy-terminus. The secondary structure of the amino-terminal residues 1–275, where the functionally important actin-binding domain of filamin has been localized [6,12], is assumed to be of random-coil conformation. In order to estimate in this amino acid stretch the secondary structure type of single residues, the prediction methods of Levin and Garnier [7] and Chou and Fasman [8] were applied.

The analysis of the ABP-280 sequence was started with the search for segments with maximum hydrophobic and amphipathic character. The most hydrophobic segments as well as the most helical amphipathic segments were found in the amino-terminal region of the protein between residues 1–275. Many highly amphipathic beta-strands were calculated for the 24 beta-sheet structure repeats (residues 275–2647). With the assumption that the crossed beta-sheet structure of repeats remains unchanged at the interaction with phospholipids, only residues 1–275 are available for a hydrophobic/amphipathic interaction with the phospholipid membrane.

Fig. 1a–d represent the structure predictions calculated for the ABP-280 primary sequence residues 1–275. The profiles in Fig. 1a and b are the average hydrophobicity and the average alpha-helical hydrophobic moment, respectively. For these calculations an amino acid window size of 19 was used. Secondary structure predictions for single amino acid residues are shown in Fig. 1c (alpha-helix, beta-strand, random-coil) and Fig. 1d (beta-turn).

The most hydrophobic region of the ABP-280 sequence is observed near residue number 137–146

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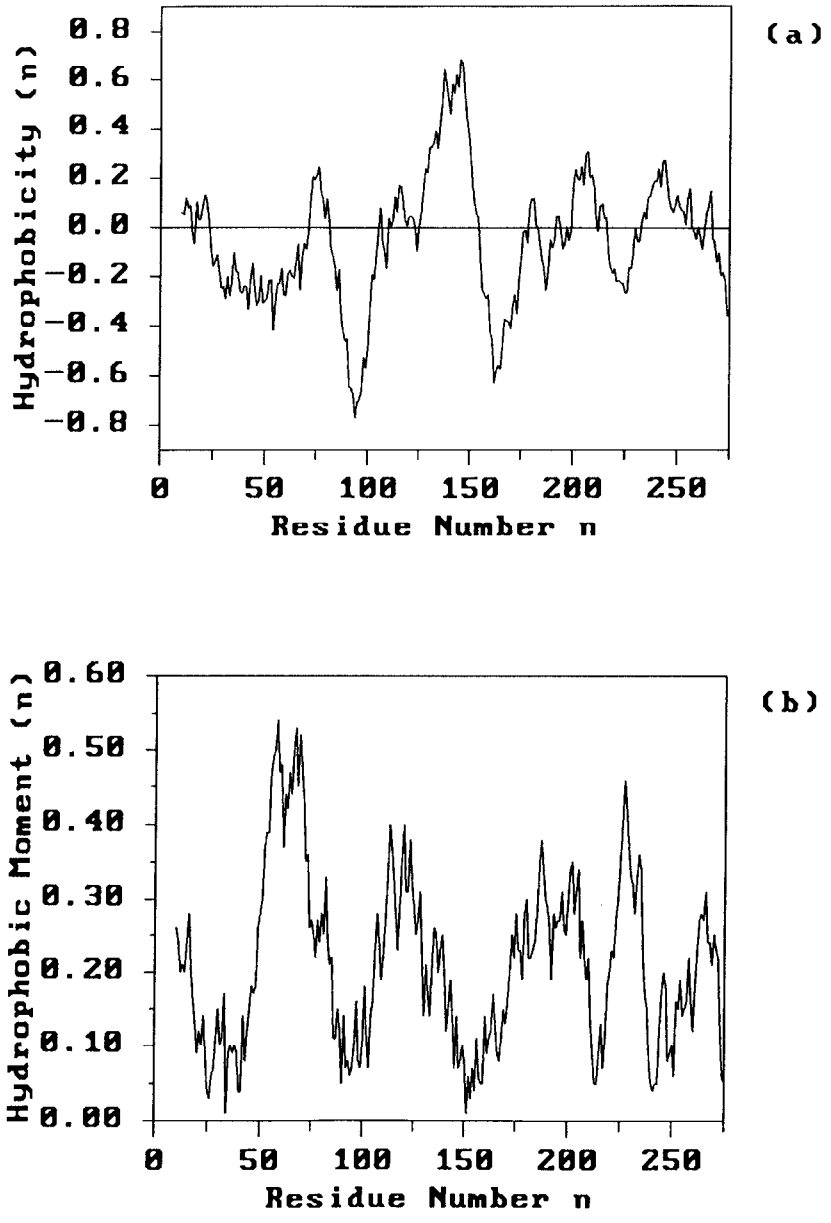

Fig. 1. Structure prediction plots for the amino-terminal end (residues 1–275) of ABP-280 with 2647 total residues. (a) The average hydrophobicity according to the method of Kyte and Doolittle [10]. Note: the increase in numbers parallels an increase in hydrophobicity. (b) The average hydrophobic moment (periodicity 3.6) according to Eisenberg et al. [11] for evaluation of amphipathic alpha-helices. (c) Predictions of secondary structure types: alpha-helical (α); beta-strand (β); random-coil (c) according to Levin and Garnier [7]. (d) Beta-turn potential according to the rules of Chou and Fasman [8]. For panels a and b the amino acid scale of Eisenberg et al. [11] was applied. The amino acid window size was 19, and the results were plotted above the middle residue of the window.

(primary sequence residues 128–155) and can be seen in Fig. 1a. The hydrophobicity plot does not differentiate between alpha-helical and beta-strand structures, nor whether a region is hydrophobic or amphipathic. Analyses of Fig. 1c reveal that the primary sequence residues 128–155 are predominantly alpha-helical. The helical hydrophobic moment shown in Fig. 1b, which is indicative of the helical amphipathic character of the sequence, has a low value and decreases continuously from residue number 137 to 146 by about 50%.

The position of the most amphipathic alpha-helix predicted for ABP-280 is shown in Fig. 1b around residue number 58–66 (primary sequence residues 49–75). The predicted secondary structure type for the amino acid region 49–75 is unambiguously alpha-helical (Fig. 1c).

The extension of the predicted helices depends on the presence of beta-turns. Normally, turns should occur at the ends of helices but not inside them. Fig. 1d shows that neither of the helices are interrupted by a turn, but both are restricted at one end by a turn. The amphipathic

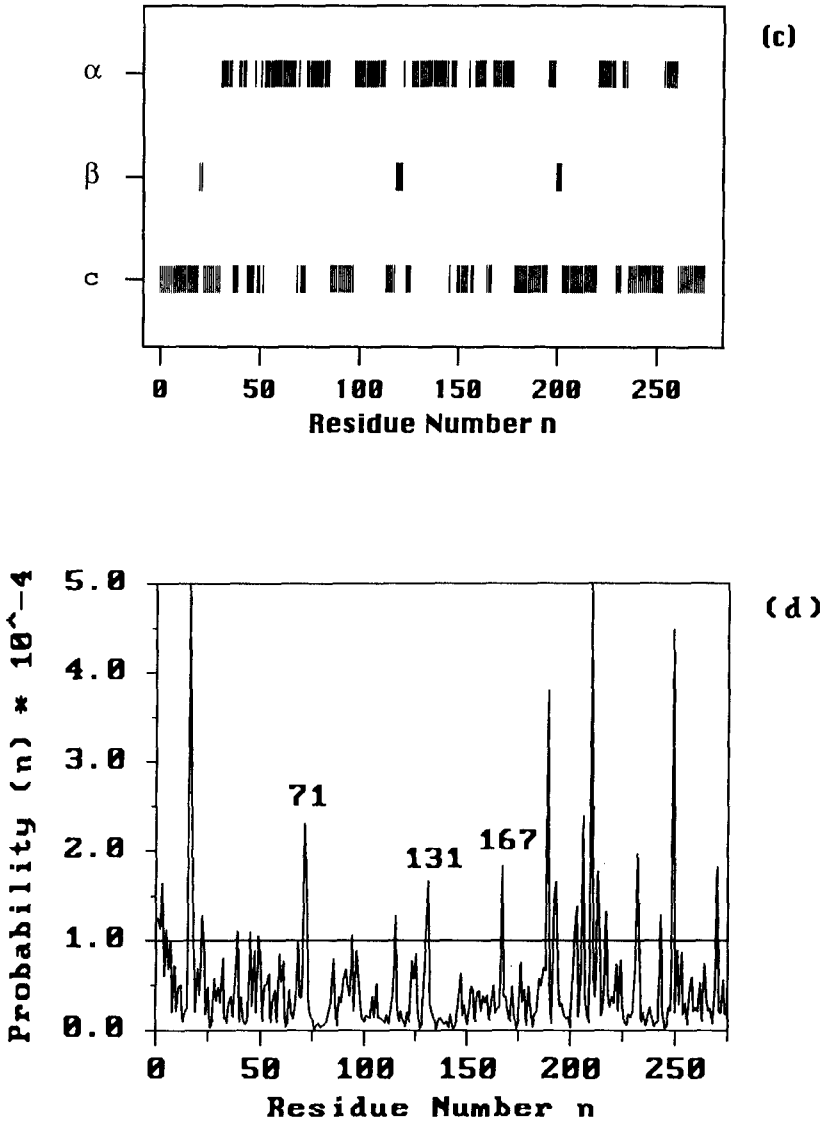

Fig. 1. (*Continued*).

helix (residues 49–75) has a breaking point at the upper end around residue 71; for the hydrophobic helix (residues 128–155) a breaking point is identified at the lower end around residue 131. Given these turns, we further expect the amphipathic and hydrophobic segments to be localized to primary sequence residues 49–71 and 131–155, respectively.

Eisenberg et al. [11] have analyzed 11-residue alpha-helices from many small proteins and peptides. They plotted the average hydrophobic moment of each 11-residue helix as a function of its average hydrophobicity and observed different patterns for globular, surface and transmembrane proteins. We used their methods to classify the two helices found for ABP-280. First, the sequence of each helix was reduced to a series of 11-residue helices. Then the hydrophobicity and helical hydrophobic moment of each 11-residue fragment was calculated, and the resulting values were plotted on the original Eisenberg diagram [11]. According to this procedure the amphipathic segment (residues 49–71; 12 fragments) is identified as a globular helix but with a high surface-seeking tendency. The hydrophobic segment (residues 131–155; 15 fragments) behaves like the combination of a surface and a transmembrane helix, with the helix character changing from the beginning to the end of the sequence from surface-seeking to transmembrane. The increasing hydrophobic strength towards the end of the sequence agrees with the predicted decrease in amphipathy (cf. Fig. 1b).

In summary, the application of computational prediction methods to the amino acid sequence of ABP-280 suggests two alpha-helical segments in the actin-binding

domain of the protein (residues 1–275) which could attach/anchor the protein to/in lipid membranes. Following Eisenberg et al. [11], the first segment (primary sequence residues 49–71) is classified as a globular helix with high surface-seeking tendency. This segment could bind to a membrane surface similar to the amphipathic apo-lipoproteins [13]. The other segment (primary sequence residues 131–155) is predominantly classified as a transmembrane helix. This segment appears as the most probable stretch of the ABP-280 sequence for hydrophobic membrane penetration. According to Eisenberg et al. [11] membrane penetration requires an average hydrophobicity of 0.68 or more. As shown in Fig. 1a at residue number 145 (primary sequence residues 136–154) the average hydrophobicity has a value of 0.69.

These structure predictions provide further grounds for the speculation that filamin could couple the cytoskeleton to the lipid membrane, and a comprehensive experimental study is presently being conducted to verify these assumptions.

Acknowledgements: This work was supported by DFG Grant Is 25/7-1 and NATO CRG 940666.

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