Computer-assistant prediction of phospholipid binding sites of caldesmon and calponin

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Abstract The primary structure of smooth muscle caldesmon and calponin was screened for the presence of amphiphilic α-helices which can participate in the formation of protein–lipid contacts. Only one caldesmon segment (residues 645–660) having a predominantly α-helical structure and high hydrophobic moment satisfies all criteria for a surface-seeking helix and is predicted to be involved in the caldesmon–phospholipid interaction. This prediction agrees with experimental results indicating that one of the caldesmon–phospholipid binding sites is located in the sequence 628–658 [Bogatcheva et al. (1994) FEBS Lett. 342, 176]. Two segments of calponin (residues 45–55 and 85–95) exhibit high hydrophobic moments and the sequence 85–95 is characterized by a high probability of α-helix formation. This may suggest that at least one of these segments could facilitate the calponin–phospholipid interaction and that calponin, as with many other actin binding proteins, is able to interact with membranes.

Key words: Caldesmon; Calponin; Phospholipid; Sequence analysis; Structure prediction; Calmodulin

1. Introduction

Caldesmon and calponin are actin- and calmodulin-binding proteins [1–3]. Both caldesmon and calponin play important structural roles and seem to be involved in the regulation of cell motility and smooth muscle contraction [1–3]. Recently published data indicate that caldesmon takes part in receptor capping [4] and exocytosis [5]. These findings correlate with the fact that under in vitro conditions caldesmon interacts with phospholipids [6–8]. This interaction depends on ionic strength, caldesmon phosphorylation and calmodulin presence [6–8].

No experimental evidence for a calponin–phospholipid interaction is available. However, it has been shown that calponin is very often co-localized with caldesmon on actin filaments just under the cellular membrane of platelets [9]. Calponin was also found in adhesion plaques and dense bodies of smooth muscle cells [10]. These data indicate that calponin is often located close to the cell membrane. Taking into account these facts and the ability of many actin binding proteins to interact with phospholipids [11], one may suppose that calponin also possesses phospholipid-binding properties.

In this paper, we used computer-assisted prediction methods for the determination of phospholipid binding sites as a first step in the investigation of a calponin–membrane interaction. We applied the same prediction methods for determination of phospholipid binding sites of caldesmon and found that the computer-generated data correlate well with our recently published experimental results [12]. This means that the prediction methods can be successfully used for localization of phospholipid binding sites.

2. Materials and methods

The primary structure of smooth muscle caldesmon [13] and calponin [14] was used in structure predictions by computer simulation. The secondary structure was analyzed by the method of Garnier et al. [15], using the PCGENE package (subprogram GARNIER). β-turns were predicted by applying the methods of Chou and Fasman [16] (subprogram BETATURN of the PCGENE package).

In order to identify highly hydrophobic or amphiphilic segments within caldesmon and calponin structures, plots for the average hydrophobicity [17] and hydrophobic moments [18] were constructed. The normalized scale of Eisenberg et al. [18] was used as the hydrophobicity scale for the amino acids. These calculations were performed by the subprograms SOAP and HELIXWEEL of the PCGENE package. The hydrophobic moments of α-helices were calculated assuming a periodicity in the hydrophobicity of 3.6 residues. An amino acid window size of 11 was used, and the results for each fifth window were plotted against the residue number or were used for constructing the Eisenberg et al. [18] plot (hydrophobic moments against hydrophobicity).

3. Results and discussion

It is supposed that protein segments interacting with membranes should satisfy a number of criteria [18,19]. When analyzing a number of proteins and peptides, Eisenberg et al. [18] plotted the average hydrophobic moment (μ) (i.e. amphiphilicity) against the average hydrophobicity (H) and found three regions on this map. One region of very high hydrophobicity includes transmembrane protein segments. The second (so-called globular) region contains the segments interacting with the solvent, and the third (so-called surface-seeking) region contains highly amphiphilic segments tending to attach to the phospholipid surface. The arbitrary line dividing the second and the third group of segments can be described as $\mu = (-0.392 \times H) + 0.603$ [18]. This means that the segments with $(\mu + 0.392 \times H)$ larger than or equal to 0.603 are classified as the surface-seeking segments and have properties similar to those of the typical phospholipid binding peptides mellitin and δ-hemolysin [18]. In addition, the phospholipid binding segments should have a high probability of α-helix formation and should not be interrupted by β-turn-forming regions [18,19]. Taking into account all these criteria we tried to predict the location of phospholipid binding sites of caldesmon and calponin.

Caldesmon is a very hydrophylic protein. Its hydrophobicity plot is mainly negative (see Fig. 1A) and the average hydropathy (GRAVY) is equal to −16.34. None of the 21-residue seg-

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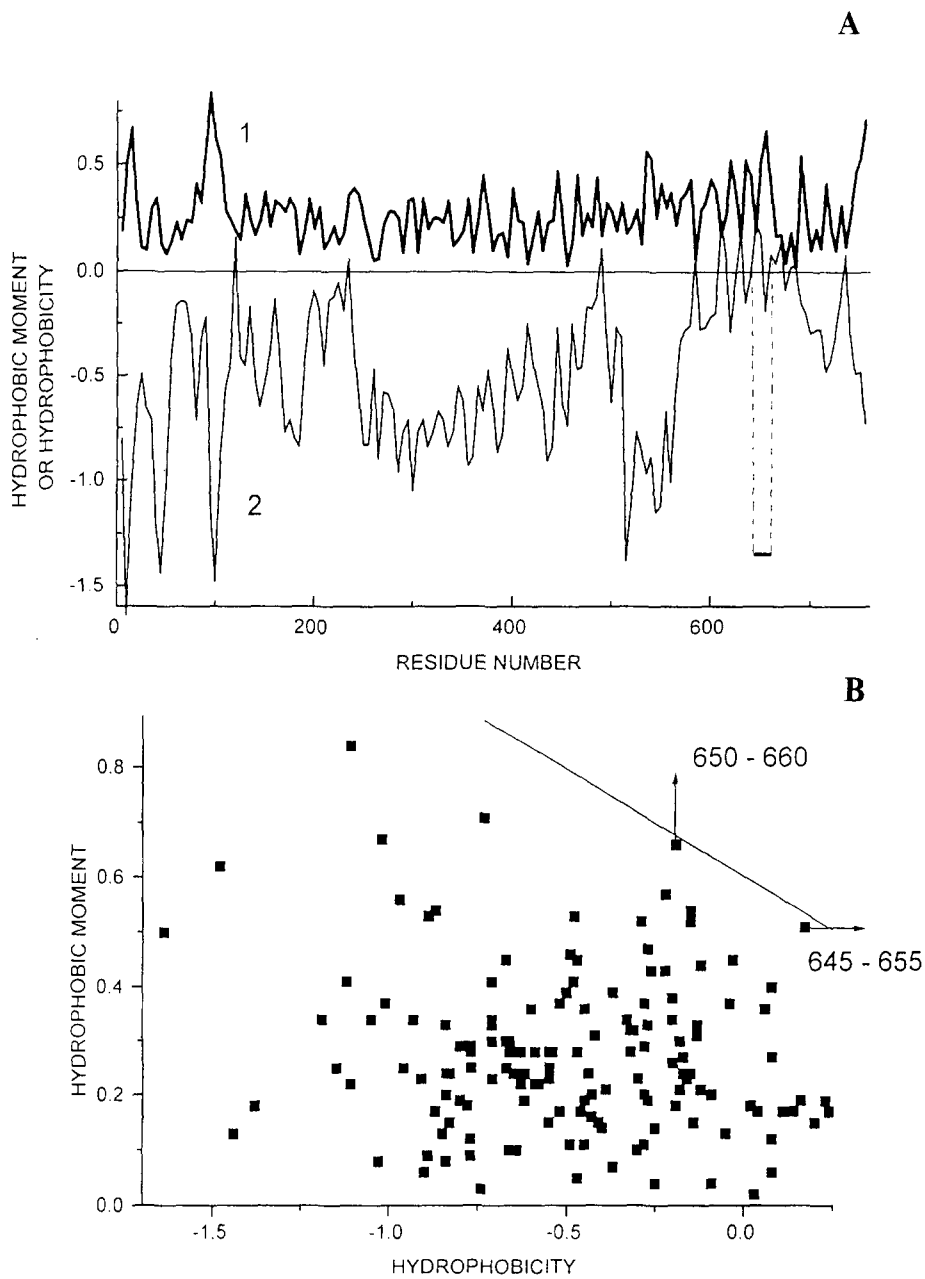

Fig. 1. Structure prediction plots for smooth muscle caldesmon. (A) Average hydrophobic moment according to Eisenberg et al. [18] (periodicity 3.6) (curve 1) and the average hydrophobicity according to Kyte and Doolittle [17] (curve 2). The amino acid window size was 11 and each fifth window was plotted. (B) Hydrophobic moment plot for each fifth 11-member segment of caldesmon. The points close to the line $\mu = (-0.392 \times H) + 0.603$ represent surface-seeking segments [18] with a high tendency to interact with membranes. Segments 650–660 and 645–655 are indicated by arrows.

ments of this protein has hydrophobicity values greater than or equal to 0.42. This means that, according to Eisenberg et al. [18], caldesmon does not contain transmembrane segments.

There are three short stretches of caldesmon having a positive average hydropathy (residues 115–125, 230–240, 485–495), but the hydrophobic moments of these fragments are rather low (0.19, 0.36 and 0.21, respectively) (Fig. 1A). Therefore these stretches can hardly form phospholipid binding sites. The most hydrophobic region is formed by residues 610–680. This part of the molecule also contains some segments with high hydrophobic moments (Fig. 1A). We constructed the plot of Eisenberg et al. [18] for caldesmon and found that the fragment restricted by residues 649–659 has the value of $(\mu + 0.392 \times H)$ equal to 0.607, whereas for three other segments (645–655, 650–660, 651–661 and 652–662) this value is equal to 0.58 (the data for segments 640–650 and 645–655 are shown on Fig. 1B). Thus, these segments satisfy one of the above-mentioned criteria for the surface-seeking sequences and can be involved in the formation of the phospholipid binding site. This suggestion is correct only if these fragments exhibit a high tendency of α-helix formation. Indeed, the method of Garnier et al. [15] predicts that residues 648–659 are helix-forming. This α-helix

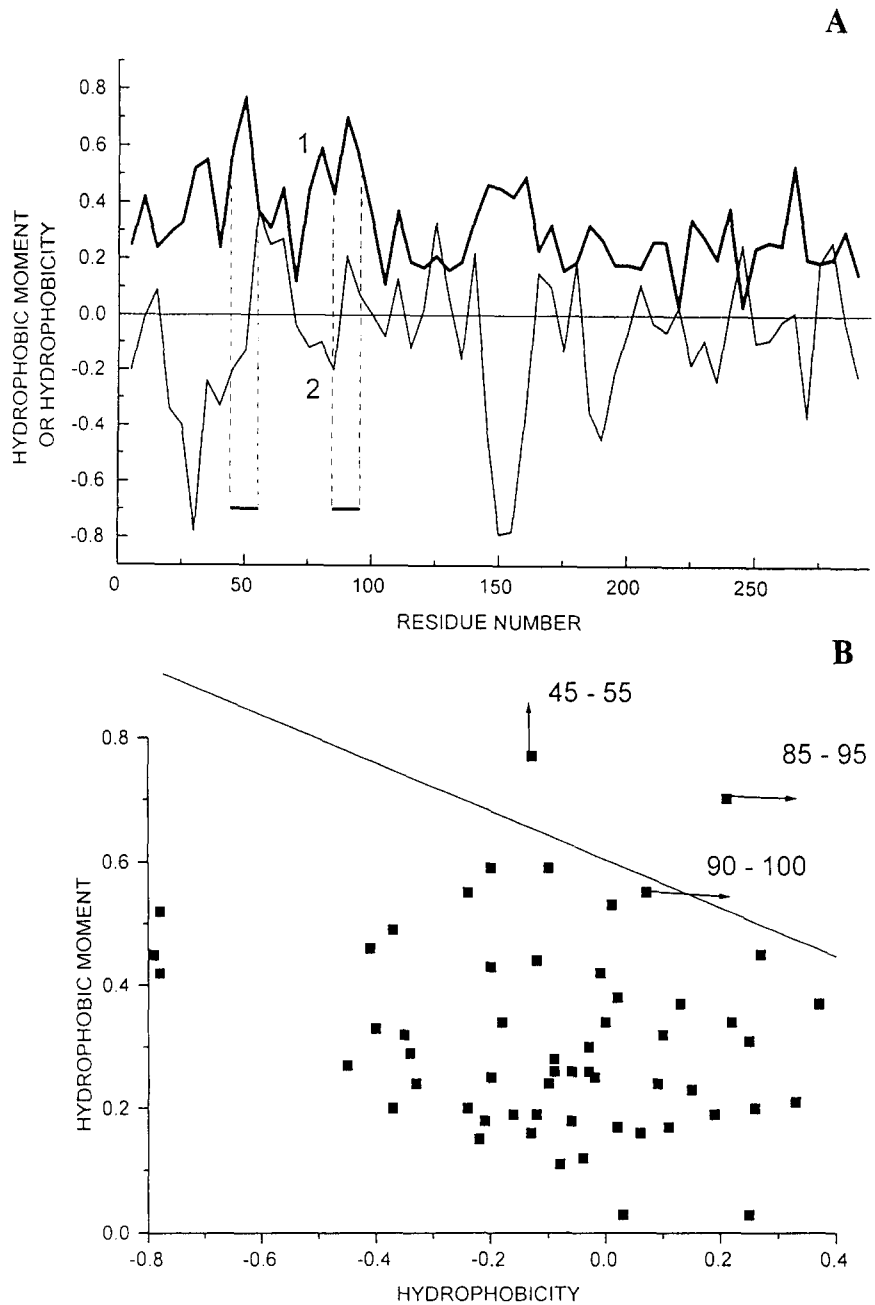

Fig. 2. Structure prediction plots for smooth muscle calponin. (A) Average hydrophobic moment according to Eisenberg et al. [18] (curve 1) and the average hydrophobicity according to Kyte and Doolittle [17] (curve 2). The amino acid window size was 11 and each fifth window was plotted. (B) Hydrophobic moment plot for each fifth 11-member segment of calponin. The points above or close to the line $\mu = (-0.392 \times H) + 0.603$ represent surface-seeking segments [18] with a high tendency to interact with membranes. Segments 45–55, 85–95 and 90–100 are indicated by arrows.

seems not to be interrupted by a β-turn since the β-turn probability for residues 630–660 is less than 1×10^{-4}. Thus we may conclude that the predominantly α-helical and highly amphiphilic segment of caldesmon formed by residues 649–662 can be involved in the caldesmon–phospholipid interaction.

Recently we tried to map the phospholipid binding sites of caldesmon using caldesmon fragments obtained by bacterial expression [12]. Our data indicate that a phospholipid binding site (or sites) is located between residues 626 and 710 of caldesmon and includes the sites of the caldesmon–calmodulin interaction and the sites of caldesmon phosphorylation by different protein kinases. The predictions obtained by the method of Eisenberg et al. [18] completely correlates with our experimental results. One of the calmodulin binding sites (site A, residues 658–668) overlaps with the predicted phospholipid binding site (residues 649–662), and Ser-657 and Ser-667, phosphorylated by protein kinase C and Pro-directed protein kinases, are inside or close to the predicted phospholipid binding segment. Thus, the predictions obtained by the method of Eisenberg et al. [18] correlate well with experimental results.

Encouraged by this fact we used the same approach to define putative phospholipid binding sites in the calponin structure.

Calponin is more hydrophobic than caldesmon (Fig. 2A). The average hydropathy of calponin (GRAVY) is −6.79. This protein does not contain 21-residue segments with a hydrophobicity greater than 0.42, i.e. it does not contain membrane-spanning α-helica segments [18].

There are two fragments of the calponin structure where both hydrophobicity and hydrophobic moments are rather high (Fig. 2A). The first segment (residues 43–56) contains four 11-member fragments with a ($\mu + 0.392 \times H$) value higher than 0.603, and for the segments 42–52, 47–57 and 48–58 its value equals 0.56–0.59 (the data for segment 45–55 are presented in Fig. 2B). Thus if the segment containing residues 42–58 is α-helical, then it can be involved in the protein–phospholipid interaction. Only six residues (52–57) tend to form an α-helix, whereas residues 45–51 and 58–66 are in an extended conformation [15]. There are three points with a high ($>1 \times 10^{-4}$) β-turn probability within the stretch 42–58 [16]. These properties will decrease the probability of segment 42–58 interaction with phospholipids.

The second region of high hydrophobicity and hydrophobic moment is formed by residues 85–99 of calponin. This region contains five 11-member windows with a ($\mu + 0.392 \times H$) value in the range of 0.62–0.78 (the data for the segments 85–95 and 90–100 are presented in Fig. 2B). For two adjacent 11-member segments (residues 90–100 and 91–101), this parameter equals 0.56–0.58. Residues 89–95 tend to form an α-helix, and residues 85, 86, 96 and 100 tend to have an extended conformation [15]. Moreover, the fragment 75–100 has a rather low (less than 1×10^{-4}) probability of β-turn formation. Thus, the segment of calponin containing residues 85–100 is characterized by a high probability of amphiphilic α-helix formation and is moderately hydrophobic. Therefore this part of the molecule can be classified as a surface-seeking helix, which can be involved in the protein–phospholipid interaction. Our future experiments will be directed to the experimental verification of the suggestion that calponin interacts with phospholipids.

If our predictions are correct and calponin indeed is able to interact with phospholipids, then the phospholipid binding site is located inside or close to the calmodulin-, tropomyosin- and actin-binding sites of calponin [3,20]. In this respect, the location of the putative phospholipid binding site of caponin seems to be similar to that of caldesmon. As already mentioned, the phospholipid binding site of caldesmon is also located close to calmodulin-, tropomyosin- and actin-binding sites [1,21,22]. This coincidence may mean that the amphiphilic α-helices can be involved both in protein–protein and protein–phospholipid interactions and that competitive binding can provide for regulation of both types of interaction.

Summing up, analyses of hydrophobicity and hydrophobic moments introduced by Eisenberg et al. [18] predicts correctly the location of the phospholipid binding sites of caldesmon. This does not mean that all phospholipid binding sites will be correctly predicted by this approach. For example, our attempt to use this approach for the so-called A-box segment of the membrane-binding proteins (such as fodrin or myosin IB) [23] was unsuccessful. Although the data of Janmey et al. [24] indicate that villin residues 111–153 are involved in interaction with phosphoinositides, the method of Eisenberg et al. [18] failed to predict this interaction. This means that the structure of the sites involved in the protein–phospholipid interaction is very diverse and is not restricted to amphiphilic α-helices. Thus, the absence of strongly amphiphilic α-helices does not mean that the protein is unable to interact with phospholipids. On the other hand, the presence of an amphiphilic α-helix may be indicative of possible interactions of a given protein with phospholipids. Our computer analyses indicate that there are amphiphilic α-helices in the structure of calponin. This suggests that calponin is able to interact with phospholipids and that the phospholipid binding site is formed by residues 85–99. Present experiments are aimed at verification of this assumption.

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